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The Effect of Nitrilotriacetic Acid (NTA) on the Structure and Functioning of Aquatic Communities in Streams

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16. ABSTRACT
Communities established in microcosms and ecosystem streams in a greenhouse were exposed to .02-2 mg/1 NTA, a range including most expected environmental levels. Higher concentrations were used in some laboratory and screening experiments. NTA at 2 and 20 mg/1 had only slight effects on algal community structure and function and 2 mg/1 protected organisms from the toxic effects of approximately 100 μg Cu^{++}/1. Protection from the toxicity of 30 μg Cu^{++}/1 was also obtained in a 3 month experiment conducted in ecosystem streams with natural sediments and more complex communities. NTA at 2 mg/1 did not result in increased concentrations of Zn, Fe, Mn, Mg or Cu in algae, Anacharis, Lemna, Planaria, and Tubifex spp.; Zn concentrations in algae, Anacharis and Lemna were frequently reduced; accumulation of added Cu by tubificids was not prevented by NTA. Bacterial communities adapted to 0.02-20 mg NTA/1 and degraded the compound under aerobic conditions. Glucose metabolism of non-NTA degrading bacterial communities measured in vitro was protected from metal ion toxicity when Cu,Zn,Cd,Mi,Pb, and Hg were complexed with NTA. Glucose metabolism by NTA degrading bacteria was inhibited, however, presumably as a result of a release of Cd,Cu, and Zn ions from concomitant NTA degradation. Substantial extraction of metals from sediments occurred at 10^{-3}M (200 mg/1) but not at 10^{-5} or 10^{-7} M NTA. Although NTA was relatively resistant to chlorination, IDA, in concentrated solution, reacted rapidly with aqueous chlorine to produce an unstable product with oxidizing properties, presumably N-chloro IDA. Both NTA and IDA could be photooxidized in the presence of a sensitizer, but the reactions were very slow.

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THE EFFECT OF NITRILOTRIACETIC ACID (NTA) ON THE STRUCTURE AND FUNCTIONING OF AQUATIC COMMUNITIES IN STREAMS

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FOREWORD

The Great Lakes with their large volumes and long retention times present a unique set of problems when the introduction of new chemicals is contemplated. Sometimes the substitution of one chemical for another in an effort to improve water quality results in additional problems.

This study provides some insight into the ecological consequences of discharging a substitute chemical. The problems associated with the discharge of phosphorus have long been documented, however, careful research is required prior to the wide spread use of a substitute.

J. David Yount
Acting Director
Environmental Research Lab.-Duluth

ABSTRACT

Studies were conducted to determine some of the effects of nitrilotriacetic acid (NTA) on organisms characteristic of a natural stream, measure its degradation by these organisms, and assess some of its possible abiotic chemical reactions under environmental conditions with particular emphasis on chelation properties. Near-natural communities were established in microcosms and ecosystem streams in a greenhouse and exposed to NTA concentrations of 0.02 to 2 mg/l (10^{-7} - 10^{-5} $\underline{M}$), a range including and exceeding most expected environmental levels. Higher concentrations were used in some laboratory and screening experiments.

NTA at 2 and 20 mg/l had only slight effects on algal community structure and function, and 2 mg/l protected organisms from the toxic effects of approximately 100 µg Cu^{++}/l.

Protection from the toxicity of 30 µg Cu^{++}/l with 2 mg/l NTA was also obtained in an experiment lasting 3 months conducted in ecosystem streams containing natural sediments and more complex communities. In this experiment, exposure to 2 mg NTA/l did not result in increased concentrations of Zn, Fe, Mn, Mg, or Cu in algae, *Anacharis*, *Lemna*, *Planaria*, and *Tubifex* spp. Zinc concentrations in algae, *Anacharis*, and *Lemna* were frequently reduced. The accumulation of added Cu by tubificids was not prevented by NTA.

Bacterial communities adapted readily to the presence of 0.02-20 mg NTA/l and degraded the compound under aerobic conditions. Glucose metabolism of non-NTA degrading bacterial communities was protected from metal ion toxicity when Cu, Zn, Cd, Ni, Pb, and Hg were complexed with NTA. Glucose metabolism by NTA degrading bacteria was inhibited, however, presumably as a result of release of Cd, Cu, and Zn ions from concomitant NTA degradation.

Substantial extraction of metals from sediments occurred at 10^{-3} $\underline{M}$ (200 mg/l) but not at 10^{-5} or 10^{-7} $\underline{M}$ NTA.

Although NTA was relatively resistant to chlorination, IDA, in concentrated solution, reacted rapidly with aqueous chlorine to produce an unstable product with oxidizing properties, presumably N-chloro-IDA. Attempts to isolate the product were not successful.

Both NTA and IDA could be photooxidized in the presence of a sensitizer, but the reactions were very slow.

This report was submitted in fulfillment of Grant No. 801951 to the Academy of Natural Sciences of Philadelphia under the sponsorship of the U.S. Environmental Protection Agency. Work was completed as of January 31, 1977.

CONTENTS

Abstract iv

Figures viii

Tables xi

Acknowledgment xv

1. Conclusions and Recommendations 1
2. Introduction 4
3. Literature Review 6
4. Methods and Procedures 16
5. Results and Discussion 28

References 65

FIGURES

Number Page

1 Structures of NTA (R = CH_2COOH) and some related compounds: IDA, R = H: EDTA, R = $N(CH_2COOH)_2$: MIDA, R = CH_3 6

2 Chelates of calcium with tripolyphosphate and nitrilotriacetate anions 7

3 NTA analytical methods -- ranges and limitations . 8

4 GC separation of butyl ester of twenty-five carboxylic acids 72

5 Standard curve for NTA gas chromatographic analysis 73

6 Effect of NTA on solubilization of copper from three stream sediments 74

7 Effect of NTA on solubilization of iron from three stream sediments 75

8 Effect of NTA on solubilization of manganese from three stream sediments 76

9 Effect of NTA on solubilization of zinc from three stream sediments 77

10 Mineralization ($^{14}CO_2$ evolution) of NTA by cell suspensions of a *Pseudomonas* sp. known to degrade NTA 78

11 Semi-log plot of NTA degradation rate ($\bar{x} \pm$ s.d.) by bacterial populations at 9°C 79

12 Semi-log plot of number ($\bar{x} \pm$ s.d.) of NTA degrading bacteria on coverslips exposed to NTA at the indicated concentrations 80

FIGURES (cont'd)

Number page

13 Semi-log plot of NTA degradation rate $(\bar{x} \pm s.d.)$ by bacterial populations at 18°C 81

14 Semi-log plot of number of NTA degrading bacteria $(\bar{x} \pm s.d.)$ on coverslips exposed to NTA at approximately the indicated concentrations 82

15A 15B 15C Changing concentrations of NH_3-N, NO_2-N, and NO_3-N resulting from the degradation of NTA in 87 l microcosms 83

16 Changing concentrations of ^{14}C-NTA in an 87 l microcosm system resulting from dilution (Theoretical curve) and both dilution and bacterial decomposition (Empirical curve) 85

17 Incorporation of ^{14}C glucose by washed cells from a culture of naturally occurring bacteria in the presence of added metal ions or NTA-metal chelates 86

18 Incorporation (I = ○ ●, open = NTA exposed, closed = control) and mineralization (M = △ ▲, open = NTA exposed, closed = control) of ^{14}C glucose by suspensions of washed cells harvested from cultures of mixed natural populations 87

19 Concentration of copper $(\bar{x} \pm s.d.)$ in algal communities from ecosystem streams through time . . . 88

20 Concentration of iron $(\bar{x} \pm s.d.)$ in algal communities from ecosystem stream through time . . . 89

21 Concentration of zinc $(\bar{x} \pm s.d.)$ in algal communities from ecosystem streams through time . . . 90

22 Concentration of manganese $(\bar{x} \pm s.d.)$ in algal communities from ecosystem streams through time 91

23 Concentration of magnesium $(\bar{x} \pm s.d.)$ in algal communities from ecosystem streams through time 92

24 Concentration of copper $(\bar{x} \pm s.d.)$ in *Anacharis* from ecosystem streams through time 93

FIGURES (cont'd)

Number Page

25 Concentration of iron ($\bar{x}$ ± s.d.) in *Anacharis* from ecosystem streams through time 94

26 Concentration of zinc ($\bar{x}$ ± s.d.) in *Anacharis* from ecosystem streams through time 95

27 Concentration of manganese ($\bar{x}$ ± s.d.) in *Anacharis* from ecosystem streams through time 96

28 Concentration of magnesium ($\bar{x}$ ± s.d.) in *Anacharis* from ecosystem streams through time 97

29 Concentration of copper ($\bar{x}$ ± s.d.) in *Lemna* from ecosystem streams through time 98

30 Concentration of iron ($\bar{x}$ ± s.d.) in *Lemna* from ecosystem streams through time 99

31 Concentration of zinc ($\bar{x}$ ± s.d.) in *Lemna* from ecosystem streams through time 100

32 Concentration of manganese ($\bar{x}$ ± s.d.) in *Lemna* from ecosystem streams through time 101

33 Concentration of magnesium ($\bar{x}$ ± s.d.) in *Lemna* from ecosystem streams through time 102

34 Concentration of copper ($\bar{x}$ ± s.d.) in *Planaria* from ecosystem streams through time 103

35 Concentration of iron ($\bar{x}$ ± s.d.) in *Planaria* from ecosystem streams through time 104

36 Concentration of zinc ($\bar{x}$ ± s.d.) in *Planaria* from ecosystem streams through time 105

37 Concentration of manganese ($\bar{x}$ ± s.d.) in *Planaria* from ecosystem streams through time 106

38 Concentration of magnesium ($\bar{x}$ ± s.d.) in *Planaria* from ecosystem streams through time 107

TABLES

Number

Page

1 Frequency of determination of water chemical parameters during algae experiments 108

2 Relative retention times (RRT) and relative molar responses (RMR) of n-butylesters of twenty-five carboxylic acids 109

3 Evaluation of GC analytical procedure using carboxyl-labeled ^{14}C-NTA 110

4 Chelation of metals from sediments by 10^{-3} M NTA (191 ppm) 111

5 NTA determinations for the period November 14, 1974 - February 13, 1975 112

6 NTA determinations for July 4 - September 2, 1975 experiment 113

7 Water chemistries for the test chambers (November 14, 1974 - February 13, 1975) 114

8 Water chemistries for the experimental boxes (June 20, 1975 - September 3, 1975) 115

9 Metals in biomass (mg cation/g Dry Weight) November 1974 - February 1975) 116

10 Metals in algal biomass - mg cation/gm July - August 1975 117

11 Slide observations, November 14 - December 10, 1974 - End of seeding 118

12 Biomass, chlorophyll and ^{14}C determination (November 14, 1974 to February 13, 1975) 122

13 Detailed analysis of diatom populations by the truncated normal curve technique 123

TABLES (cont'd)

Number Page

14 Distribution of the more common diatom species . . . 124

15 Microscopic examination of communities 125

16 Analysis of diatom population using truncated normal curve factors 126

17 Distribution of the more common diatom species . . . 127

18 Effects of NTA on algal communities 128

19 Water chemistries for the experimental boxes (March 14 - April 15, 1975) 129

20 Determinations for NTA and Cu during the period March 12 - April 15, 1975 130

21 Microscopic examination of communities 131

22 Detailed analysis of diatom populations by the truncated normal curve technique 132

23 Distribution of the more common species 133

24 Biomass, chlorophyll and ^{14}C determination 134

25 Metals in algal biomass (mg cation/g) March - April 1975 135

26 NTA concentration in microcosms March 12 - April 24, 1975 136

27 NTA degradation by bacterial populations exposed to different NTA concentrations for four weeks 137

28 NTA concentration in microcosms July 2 - August 14, 1975 138

29 Effect of cadmium, zinc, lead, and nickel ions and NTA-chelates of the same on the metabolism of ^{14}C glucose by heterotrophic bacteria from White Clay Creek 139

30 Effect of copper ions and copper-NTA chelates on the metabolism of ^{14}C glucose by heterotrophic bacteria from White Clay Creek 140

TABLES (cont'd)

Number Page

31 Effect of 4 mg/l NTA (probably present as Ca or Fe chelates) on metabolism of ^{14}C glucose by heterotrophic bacteria from White Clay Creek 141

32 Effect of cadmium, zinc, and copper ions and NTA chelates of the same on metabolism of ^{14}C glucose by NTA degrading isolates 142

33 Effect of copper, cadmium, zinc, and mercury ions and NTA-metal chelates on microbial metabolism . . . 143

34 Concentration of selected chemical species in ecosystem stream water; July 13-October 6, 1976 . . . 144

35 Concentrations of NTA and copper in ecosystem stream water; July 13-October 6, 1975 145

36 Comparison of concentration of Cu, Fe, Zn, Mn and Mg in algal biomass from ecosystem streams at a given sampling time 146

37 Comparison of concentration of Cu, Fe, Zn, Mn and Mg in *Anacharis* from ecosystem streams at a given sampling time 147

38 Comparison of concentration of Cu, Fe, Zn, Mn and Mg in *Lemna* from ecosystem streams at a given sampling time 148

39 Comparison of concentration of Cu, Fe, Zn, Mn and Mg in *Planaria* from ecosystem streams at a given sampling time 149

40 Grand mean of metal concentrations in Tubificid worms from collections 2-8 150

41 Cumulative mean concentration of metal ions in the water in ecosystem streams; August 11 - October 6, 1976 151

42 Concentration factors (metals in biomass of indicated samples/metal in water) 152

43 Concentration factors (metal in *Planaria*/metal in algae) 153

44 Concentrations of copper and NTA in microcosms for sediment studies 154

TABLES (cont'd)

Number | Page

45 Concentration of copper (ng/g) in sediments exposed to copper or copper/NTA 155

46 Concentration of iron, manganese, and zinc (all in µg/g) in sediments on 12/17 after 65 days exposure to copper or copper/NTA 156

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SECTION 1

CONCLUSIONS AND RECOMMENDATIONS

The purpose of this study was to determine the effects of NTA on organisms in streams, and on heavy metals in sediments of the stream bed. The effects of chlorination on NTA and IDA were also examined. To accomplish this purpose, we examined effects on the metabolism of bacteria and algae. We also studied the effects of NTA on the uptake of heavy metals by organisms living in the sediments and in the water of a flowing stream.

The experiments were conducted in flowing water microcosms and ecosystem streams in which many natural conditions were reproduced. Ecological aspects of NTA exposure were emphasized. NTA concentrations ranging from 0.02 - 200 mg/l (10^{-7} - 10^{-3} $\underline{M}$) were studied. Usually, concentrations that might be found in natural habitats (0.02 - 2.00 mg/l) were used, but higher levels were occasionally used to verify that experimental conditions were appropriate when effects were not evident at lower concentrations. Unless other metal ions were deliberately added, dissolved NTA in these experiments was principally present as its calcium and iron complexes.

A comparison of various methods of analyses of NTA was made. It was found that below 2 ppm the Zinc-Zincon method produced results that averaged somewhat higher than gas chromatographic results. The mathematical relationship between the two methods was: mg/l NTA (zinc - zincon) = 1.036 x mg/l NTA (g.c.) + 0.356 (R = 0.962).

The presence of 10^{-5} $\underline{M}$ (1.9 mg/l) or less NTA did not lead to significantly higher concentrations of metal in water. At 10^{-3} $\underline{M}$ concentration of NTA (191 mg/l) there were significant increases in the water (3x - 15x). At 10^{-3} $\underline{M}$ IDA the concentration of Fe, Zn, and Cu were significantly higher in the water. In the presence of sewage inoculum, Cu and Zn concentrations dropped in 10^{-3} $\underline{M}$ NTA experiments.

Experiments with chlorinating water containing NTA indicated that even if N-chloro-IDA was formed it would not persist, as acidic and basic pathways are available for its degradation.

Natural bacterial communities degraded NTA under aerobic conditions at different seasons of the year. During the course of experiment, populations became acclimatized, and the degradation of NTA was directly correlated with temperature, length of exposure time, and concentration of NTA. NTA degradation under anaerobic conditions has been demonstrated in other studies (See Literature Review). The redox potential of the sediment may be an important determinant as to whether this will occur and additional investigations should address this correlation.

NTA degradation under aerobic conditions resulted in an increase in NH_4-N followed by bacterial oxidation of the ammonium-N to NO_2-N. Nitrate-N concentration increases were not detected in our system when NTA was present at environmentally realistic NTA levels, because NO_3-N in the stream water was already 3.0 mg/l, but in other environments the nitrate concentration may increase. The extent to which NTA usage might accelerate eutrophication through nitrogen additions has been considered minimal by other workers (See Literature Review) and has to be weighed against additions of other algal nutrients when alternative non-NTA containing compounds are used. Studies of possible effects on detritus production should be considered in future work since nitrogen supply may affect processing.

NTA had no overt effect on the metabolism of some other organic compounds (glucose, amino acids) by natural bacterial populations. NTA protected bacterial metabolism from concentrations of copper, cadmium, zinc, lead, and mercury ions that were otherwise toxic to varying degrees. However, studies with NTA-degrading isolates suggested that free ions of zinc, cadmium and copper could be released in sufficient amounts from concomitant NTA-chelate degradation to inhibit glucose metabolism under the conditions of the test. These populations were not specifically acclimatized to the heavy metals tested and this may be an important variable. In nature, sediments or other particulate and dissolved organic compounds may offer alternative binding sites for released metals but if inhibition of bacterial activity should occur, the self-purification capacity of a system would be impeded. This potential problem should be considered in future evaluations of environmental effects of NTA.

The results of the algal community experiments indicate that NTA in concentrations of 2 mg/l and 20 mg/l had no adverse effects on the structure of the diatom communities, and there was no significant change in the amount of the green algae *Stigeoclonium lubricum* or of blue-green algae. The results of the June-September and November-February were similar except that in the 20 mg/l experiments of June-September the ash free dry weight was somewhat less than in the controls. Since 2 mg/l and 20 mg/l NTA are higher than present literature indicates occur in natural bodies of water that have received NTA over considerable periods of time, these experiments indicate that no significant changes would occur in the algal communities at these or lower concentrations.

In experiments adding 2 mg/l NTA with and without the addition of 30 μg/l copper, the results indicated that the NTA chelated the copper both naturally occurring and added, so that it did not accumulate significantly in the algal cells, and that their growth and ^{14}C uptake were similar to the controls. In those experiments with only 30 μg/l of copper, the algal cells died.

In a three-month experiment conducted in ecosystem streams containing natural sediments, 2 mg NTA/l exhibited no acute or chronic toxicity to natural communities of algae, two aquatic plants (*Lemna* and *Anacharis*), or the worm genera *Planaria* and *Tubifex*. This NTA concentration also protected organisms from the toxic effects of 30 μg Cu/l when added simultaneously. From macroscopic observations, there were no significant shifts in the structure of the communities and the species composing them in the control, NTA, and NTA and copper experiment. Where 30 μg/l Cu was added, *Anacharis* and algae died, but a population of *Ulothrix* recolonized the stream after three weeks. Concentrations of Cu, Zn, Mn, Mg, or Fe in the tissues of these organisms did not increase significantly as a consequence of NTA exposure. In fact, the concentration of zinc in the biomass of algae, *Anacharis* and *Lemna* tended to be lower when NTA was present, suggesting that NTA-chelated zinc might be less available to these organisms. Study in other habitats is necessary to test the generality of this observation since chelation equilibria depend on the relative concentrations of dissolved metal ions and on competition from sorptive surfaces and other chelating substances, such as naturally occurring humic materials and secretions from organisms. The presence of NTA did not reduce the accumulation of copper added to the water column by sediments; nor did the presence of NTA prevent the accumulation of added copper by sediment-dwelling tubificid worms.

We have addressed some of the environmental aspects of NTA usage in this work. The environmental consequences of moderate NTA usage do not appear to be detrimental. If one wishes to compare the ecosystem effects of NTA with other commercial formulations thorough investigations in systems of different types are needed. Although some evidence may be gleaned from existing monitoring studies in countries where NTA usage is greater than in the United States, this should not substitute for additional comparative investigations in large scale experimental ecosystems (such as those used in this study) over long periods or detailed environmental monitoring if greater usage of NTA occurs in this country.

SECTION 2

INTRODUCTION

The purposes of these experiments were several. One was to determine the effects on selected freshwater organisms of NTA, some of its degradation products, and products that might be formed by the chlorination of NTA. Second was to determine the effects of NTA on the release of metals from sediments. Third was to study in a stream ecosystem the effects of NTA on metabolic processes of organisms representing some of the stages of energy transfer in the food web. These studies included its effect on the uptake of Cu.

Nitrilotriacetic acid (NTA) has been used in industrial processes, agriculture, and in some countries in detergent formulations. At the time this program of work was initiated, experiments had been carried out to determine the chemical characteristics of NTA, its degradation (with particular emphasis on biochemical pathways of selected isolates and rates of decomposition in sewage treatment facilities), and its acute toxicity had been reported or was being investigated (See Literature Review). Little, however, was known of its fate in receiving waters or the effects on natural communities of chronic exposure. The studies reported here were conducted to provide information concerning the possible effects of NTA on aquatic communities in streams when conditions of exposure simulated nature in many respects. Special attention was also given to the chelation and possible mobilization of metals by NTA.

It has been predicted that NTA will be present in many natural environments at low concentrations if the compound is widely used. Although estimates of discharge from sewage treatment facilities have ranged in concentration from 1-20 mg/l, most reported values fall at the lower end of this range. With a 10-1000 fold or more dilution in the receiving body of water, concentrations greater than .02 - 2 mg/l would seldom occur. The use of concentrations from .02 - 2 mg/l seemed reasonable for studying the effects in natural bodies of water.

The microbial components of a community are frequently most responsive to changes in water chemistry. Therefore, in evaluating the environmental effects of NTA in this program, emphasis was placed on the algal and bacterial populations.

In many aquatic systems, algae are the important primary producer organisms, although aquatic macrophytes may assume local importance. Algae require for growth macronutrients such as carbon dioxide, nitrate-N, ammonia-N, phosphates, some metals (Ca, Mg, K) and a host of organic and inorganic micronutrients such as vitamins and trace metals. Individual species have exacting requirements for these trace substances. The biomass of algae produced may be primarily dependent on the supply of macronutrients but the species of the algae found in a given system may be related primarily to the supply of micronutrients. For example, Patrick, Crum, and Coles (48) and Patrick, Bott, Larson (47) have shown that manganese, vanadium, nickel, and selenium at various concentrations altered the dominant algae in a community by fostering the development either of diatoms or blue-green algae. Shifts in algal communities brought about by the presence of organic substances have been shown by Rice (56), Proctor (53) and Keating (35).

Changes in micronutrient concentrations may bring about the development of communities of algae that are desirable or undesirable food species. In the former instance, efficient transfer of energy from primary producers to higher forms will be enhanced; in the latter instance, nuisance growths ("algal blooms") may accumulate. Many observations indicate that diatoms and some unicellular green algae are desirable food sources whereas filamentous greens and blue-green algae are not as desirable food sources (29,46).

In contrast to many other investigations in which the response of organisms to NTA has been studied in single species laboratory experiments of relatively short duration, the experiments reported here were designed to investigate the effects of chronic exposure of natural communities to the compound. The emphasis in all instances was on the ecological aspects of exposure rather than on the more detailed physiological or biochemical questions concerning exposure. The following aspects were selected for our study:

1. The effects of NTA and NTA-heavy metal chelates on the community structure of algae and primary productivity.

2. The effects of NTA and NTA-metal chelates on the mineralization activity of natural bacterial communities.

3. The degradation of NTA by natural bacterial communities.

4. The behavior of NTA, copper, and NTA-chelated copper in experimental ecosystem streams with particular emphasis on the fates of the various compounds, the mobilization of metals from sediments and the identification of sinks for metal ions.

Experiments under conditions as natural as possible (continuous flow, natural light and temperature regimes) and with natural communities, but in which many variables were controlled and monitored closely were performed to study the effects of NTA and NTA + metal ions at different seasons of the year.

SECTION 3

LITERATURE REVIEW

CHEMICAL PROPERTIES

Nitrilotriacetic acid (NTA, Fig. 1) is a synthetic amino acid having a tertiary nitrogen atom. Its commercial synthesis affords the trisodium salt monohydrate in a purity of 98%; the product also contains small amounts of the related compound iminodiacetic acid (IDA, Fig. 1). In the free acid form, NTA ionizes with successive loss of three protons; the reactions have pKa's of 1.92, 2.38 and 9.95. Thus, at all pH's between 3.4 and 9.0, the NTA dianion predominates (42).

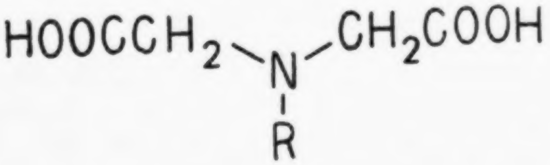


Fig. 1. Structures of NTA (R = CH_2COOH) and some related compounds: IDA, R = H: EDTA, R = $N(CH_2COOH)_2$: MIDA, R = CH_3.

Salts of NTA have extremely high water solubility and capacity for metal ion chelation. These properties (among others) have made NTA one of the prime candidates for introduction as a phosphate-replacing builder in detergents. Formulations containing NTA were, in fact, available in some parts of the United States during 1967-1970, but industry withdrew the products following expressions of concern over possible health and environmental hazards. Nevertheless, NTA continues to be used for this purpose in several other countries.

Detergent builders are used to maintain in solution alkaline-earth cations (e.g. Mg^{++}, Ca^{++}), which otherwise form insoluble curdy precipitates during the washing process. Tripolyphosphate builders form soluble, chemically stable chelates with three sites of coordination, whereas, chelates of NTA are tetrahedrally coordinated (Fig. 2).

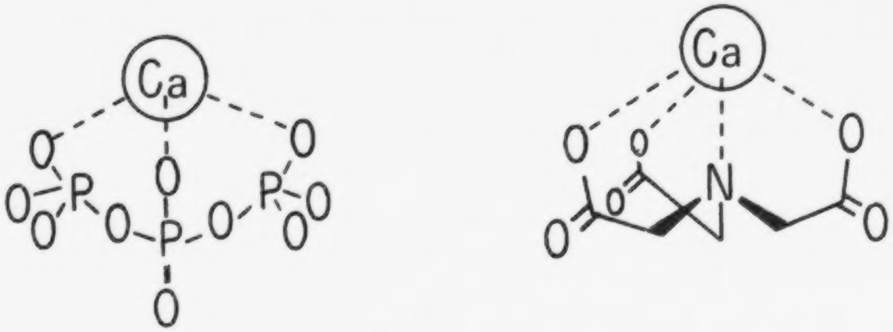


Fig. 2. Chelates of calcium with tripolyphosphate and nitrilotriacetate anions.

Equilibrium constants for the reactions of a large number of metal ions with NTA have been tabulated by Sillen and Martell (63). For all di- and trivalent cations studied, these reactions are very favorable, with equilibrium constants ranging from 6.3×10^4 (Ba^{++}) to 10^{13} (Cu^{++}). Accordingly, if the rate of metal complex formation is rapid, the level of uncomplexed NTA in almost all natural waters would be expected to be infinitesimal. The relative amounts of chelated NTA species at equilibrium would depend on the aqueous concentrations of the metal ions (Cu^{++}, Fe^{+3}, Pb^{++}, Ni^{++}, etc.) which form particularly stable complexes. Because the rates of formation of the complexes have been shown to be very fast, predictions based on equilibria probably have environmental usefulness. Childs (14) has calculated that, in Lake Ontario, the copper complex would predominate at equilibrium at concentrations of 1.5 mg NTA/l or less.

ANALYSIS OF NTA

Many methods of NTA analysis have been described. At relatively high concentrations, the colorimetric zinc-Zincon (77) method has found wide use. Below about 0.2 mg/l, polarographic (1) and gas chromatographic (6) methods appear most applicable. In addition, assays based on chemical kinetics (16), chelation (36), fluorescence (57), and ion-selective electrodes (73) have appeared, but none of these has been widely tested. The claimed sensitivity range for each method is summarized in Fig. 3. Some possible interferences in analysis of waterborne NTA are:

a) Polyvalent metal ions may complex NTA.

b) Natural chelating agents (polycarboxylic acids, polyphenols, amino acids) may interfere in methods based on chelation. In addition, IDA and other possible degradation products have chelating ability.

c) Oxidizable or reducible substances may interfere in polarographic methods.

d) Chromatographic methods must achieve essentially complete resolution of the NTA derivative from interfering substances.

e) Fluorescent, quenching, or colored substances may interfere with fluorometric or colorimetric determinations.

Analysis of NTA chelates has received little attention. A thin-layer chromatographic (TLC) method has been reported; it requires a minimum of 500 ng of the chelate, in relatively concentrated solution (55).

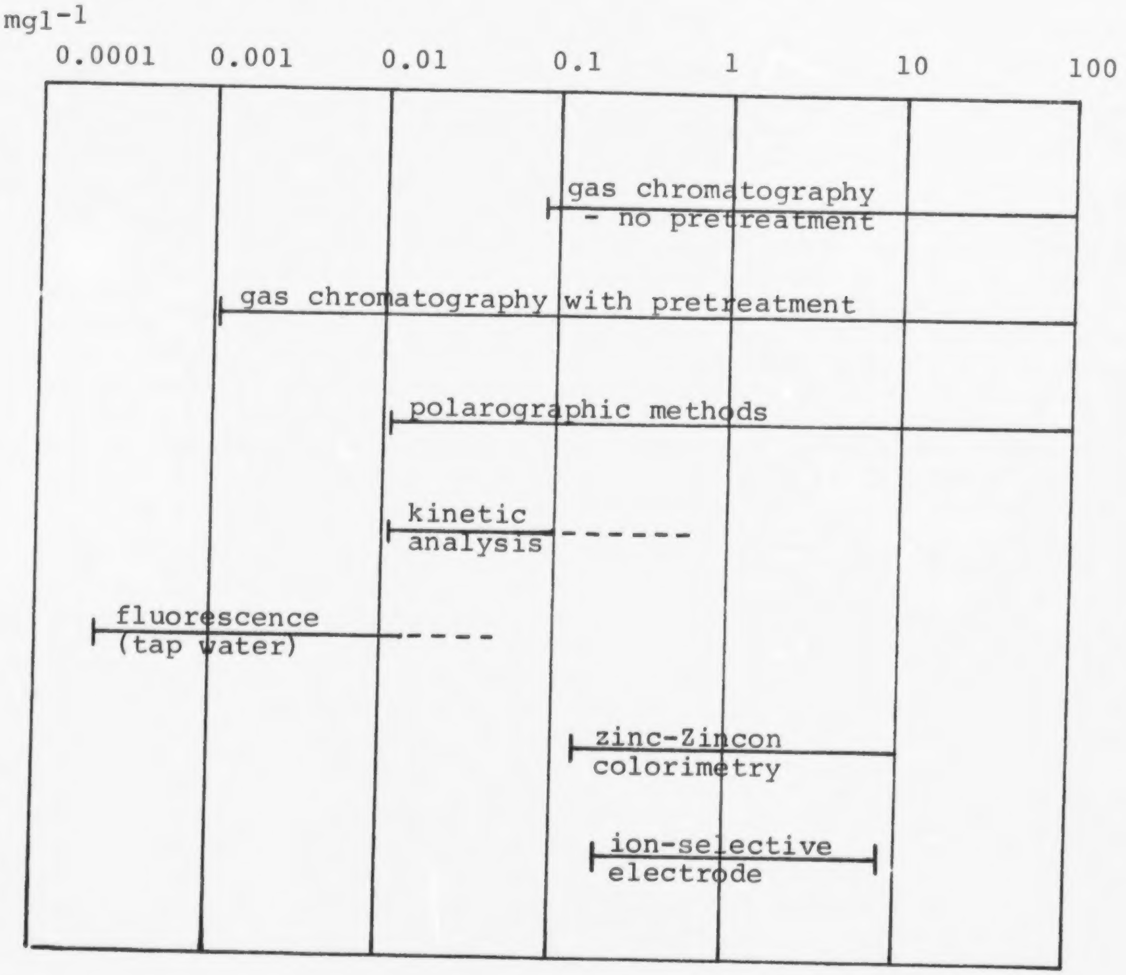


Fig. 3. NTA analytical methods — ranges and limitations.

CHEMICAL AND BIOCHEMICAL REACTIONS

Oxidation Reactions

The reactions of NTA are controlled by its high polarity and its chelating properties. Although it is a compound having considerable intrinsic (thermodynamic) stability, NTA (because of its similarity to natural amino acids) would be expected to be relatively susceptible to environmental decomposition reactions such as photooxidation or metabolic transformations by living organisms. The literature indicates that this is indeed the case. Rather harsh conditions are required to effect thermal oxidation of NTA. In the presence of a palladium catalyst and an oxygen atmosphere at 90°C, it can be converted in 92% yield to IDA (74). However, photooxidation of some NTA complexes occurs readily. Copper or iron complexes of NTA, when exposed to long wavelength ultraviolet light in the laboratory (37) or summer sunlight (66) were oxidatively transformed to IDA, formaldehyde, and CO_2. The complexes of IDA were relatively stable to further photochemical destruction. Ligand-to-metal charge transfer complexes appeared to be involved; NTA chelates of some other metal ions (Pb^{++}, Cd^{++}, Mg^{++}) were not significantly degraded.

Chelation of Metals from Sediments

It has been suggested that NTA may sequester toxic or nutrient metal ions from sediments or suspended material and possibly facilitate the transport of such metals through biological membranes. Conversely, it is conceivable that NTA could protect aquatic organisms from the toxic effects of free metal ions, or deprive them of required metallic nutrients.

Several investigations (8,13,30,50,72) of the interaction of NTA with natural sediments, employing NTA at concentrations ranging from 0.2 to 20 mg/l, have been reported in the literature. In several of the studies, the sediments were shaken with the NTA solutions. It might be argued that such a design lacks environmental relevance, and data from the studies do not agree fully. Nevertheless, certain trends may be noted. Significant increased solubilization of zinc was observed by three research groups; (72, 13,8) iron likewise appeared to be solubilized (8,13,50) although one report indicated no increased solubility (72). Manganese (72, 50), lead (30), and nickel (72) concentrations may or may not be increased by NTA. There is general agreement that levels of cobalt, chromium, copper, and several other metal ions were not significantly increased by NTA at the concentrations studied.

Interestingly, polyphosphate appeared to be about as efficient as NTA at mobilizing Fe, Zn, and Cu from sediments; EDTA (Fig. 1) was superior to both (8). Allen and Boonlayangoor (3) concluded that at 0.75 mg/l of NTA the concentration of no metal would increase more than 10% in the rivers studied. Since this concentration of NTA is much higher than 0.05 mg/l found in 95% of the Canadian rivers studied (83) they concluded that metal concentration would not measurably increase with .02 mg/l NTA.

Biodegradation

Since the early report of Forsberg and Lundquist (27) of bacteria capable of using NTA as a carbon and nitrogen source, it has been shown repeatedly that NTA is biodegradable to the inorganic end products CO_2 and NH_4^+. In pure culture, organic intermediates in the metabolism of NTA have been shown, but these have not been identified in the environment. A few biochemical studies with bacteria (primarily *Pseudomonas* species) in pure culture have been done to determine degradation pathways. The evidence suggests that NTA was oxidatively metabolized, initially with cleavage of a C-N bond, to IDA and a two-carbon fragment, probably glyoxylic acid (17,26,79). The isolates studied have been shown to oxidize the compound rapidly, reducing the concentration from 290,000 μg/l to <50 μg/l in 45 min. in one instance. Some NTA-degrading isolates did not metabolize IDA (17), while others did (26).

As noted above, NTA in solution rarely exists as the free acid, but rather as metal chelates. Concern has been expressed as to whether the heavy metal chelates of NTA could be readily degraded. Firestone and Tiedje (23) reported a series of experiments with an NTA degrading *Pseudomonas* sp. and found the organism was capable of degrading the Ca, Mn, Mg, Cu, Ni, Cd, Fe, and Na chelates at approximately the same rates if the concentration of freed metal did not become toxic. If this occurred, the addition of soil allowed continued degradation, presumably by binding free metals. The nickel chelate, however, was not degraded. However, as will be discussed below, degradation of the nickel chelate in some experiments with mixed populations in soil or water has been observed. The authors hypothesized that in chelate degradation the metal was not transported into the cell with NTA but rather was disassociated at the cell surface.

Although NTA degradation was thought originally to occur only under aerobic conditions, there are recent reports of anaerobic degradation. Enfors and Molin (20,21) reported the isolation of a facultatively anaerobic bacterium capable of metabolizing NTA under anaerobic conditions if nitrate was present in the medium.

Further possible reactions of IDA have been considered by several authors (17,51,75,82). Because it is a secondary amine, it may react by pathways unavailable to NTA. The possible formation of N-nitroso IDA by the reaction of NTA with nitrite ion has

$$(HOOCCH_2)_2NH + NO_2^- \xrightarrow{H^+} (HOOCCH_2)_2NNO + H_2O$$

attracted attention because of the known carcinogenicity of many nitrosamines. N-nitroso IDA was shown to be not degraded in river water within 52 days at concentrations of 5 of 20 mg/l (82). The compound has not been detected in the environment, although mixed soil microorganisms in culture have been reported to synthesize it from NTA and nitrate (51).

CONCENTRATION AND DECOMPOSITION IN THE ENVIRONMENT

Thayer and Kensler have reviewed monitoring studies of NTA in tap waters, sewage influent and effluent, and receiving streams (75). Analysis of 165 samples of Canadian tap water for NTA gave a mean concentration of 0.006 mg/l; most of the samples had undetectably low concentrations. The data indicate that although inputs of NTA to sewage plants were relatively high (2-118 mg/l), considerable degradation occurred within the plant; reported effluent concentrations did not exceed 4 mg/l NTA, with usual values in the 1-2 mg/l range.

Following the introduction of NTA into detergent formulations in Canada (initially at 6%, later at 15%) the NTA content of effluent from primary, trickling filter and activated sludge sewage treatment plants and of receiving streams was monitored (83). Concentrations in sewage effluent ranged from 0.011 - 10.50 mg/l (geometric means for primary, trickling filter, and activated sludge plants were 2.98, 3.22, and 0.60 mg/l respectively at 15% NTA content). In receiving streams concentrations ranged from undetectable to 3.36 mg/l (the median concentration was 0.05 mg/l and 97% of all samples contained < 0.05 mg/l. Several positive correlations between metal ion and NTA concentration were observed but the authors considered these to be the results of other factors because the total concentration of chelatable metals exceeded NTA concentration. However, it is noteworthy that the metals involved were frequently those for which NTA has high affinity (Cu, Cr, Zn, and Ni).

Although methods differed, several research groups (12, 50, 69, 77) reported essentially complete degradation in laboratory experiments with activated sludge after acclimatization periods of one to three weeks. In one study (12) primary effluent was added as well as NTA. Following acclimatization 80-85% reduction occurred at 20°C but only 25% removal took place at 5°C. Different populations were active at the two temperatures; when the temperature of the system held at 5°C was elevated to 20°C negligible degradation occurred until a new population developed.

Shumate, et al. (62) performed a field study at a treatment plant and reported 90% removal of 8 mg/l NTA added to influent waste and 75% removal when the feed concentration was 16 mg/l NTA. Because of variability, it was difficult to assess any seasonal effects on removal efficiency. Rudd and Hamilton (58) studied degradation in a model aerated sewage lagoon using an influent concentration of 14 mg/l NTA. At 15°C the efficiency of degradation was 93%; at 5°C, 47%; and at 0.5°C, 22%. In cold weather

(<5°C), 50% or more of incoming NTA might be discharged into the receiving body of water. Similar data were reported for another treatment plant; 95% removal in summer, <50% in winter (61).

Further decomposition and dilution will occur in rivers and most samples taken downstream of NTA-using communities contain undetectable amounts, with only a few reports of levels exceeding 0.1 mg/l. Warren and Malec (82) used gas chromatography to detect the degradation of NTA and postulated intermediates added to unacclimatized water from the Detroit and Meramec Rivers. Degradation of NTA was complete, and intermediates could not be detected. Only N-nitroso-IDA was not degraded; N-methyl IDA, IDA and sarcosine were all degraded, although N-methyl IDA degraded more slowly. Swisher, et al. (70) studied the biodegradation of 5 mg NTA added to river water with metal ions added to provide 0.1, 0.3, and 1.0 equivalents. The chelates of Fe, Pb, Cd, Ni, Cu, and Zn were degraded following lag times of varying duration. Others (10,31,81) have reported degradation of Ni, Cu, Cd, and Cr chelates to be inhibited. Tiedje and Mason (78), however, found that the NTA chelates of Cu, Ca, Fe, Mn, Zn, Pb and Ni, were degraded rapidly when added to soil; but the Hg and Cd chelates were degraded more slowly. Recently, degradation in a receiving stream in Ontario (61) was studied throughout the year, including periods when stream temperature was less than 3°C. Under winter conditions, NTA concentrations as high as 0.125 mg/l were found about 0.8 km downstream from the input, compared to summer levels of 0.01 mg/l or less. Evidence was obtained for biodegradation in the receiving stream at low temperatures, albeit at reduced rates.

The degradation rate of NTA in soils has been related directly to organic content of the soil, temperature, and NTA concentration (78). Degradation occurred under aerobic conditions, was greatly slowed under microaerophilic conditions, and was non-existent under anaerobic conditions (argon purged systems). However, degradation on re-exposure to oxygen was not impeded by the imposed anaerobiosis (78). Tabatabai and Bremner (71), however, have reported degradation in soils sparged with He gas.

Thus, the degradation rate in any environment will be dependent on environmental conditions such as temperature, chemical milieu, degree of aeration, and the rate of acclimatization and fluctuation of populations.

EFFECTS OF NTA ON ORGANISMS

As will be discussed below, NTA has been screened for possible toxicity against a variety of aquatic organisms including algae, invertebrates, and fish. Most reported studies have been 96 hr. bioassays for acute toxicity. Possible stimulation of algal growth has also been tested in a few instances. The molecule has been generally shown to be non-toxic to the organisms tested except at concentrations greatly exceeding those expected in natural habitats. Studies with all groups of organisms have demonstrated an inverse relationship between water hardness and toxicity. NTA complexes appear not to be acutely toxic and the compound was only toxic when present in molar excess of complexing cations. This may strongly imply that NTA toxicity is rare in nature at ordinarily expected concentrations. It should be noted that studies rarely included newly hatched or young organisms — stages that are particularly sensitive to toxicants.

Bacteria

In one study, growth of a strain of the bacterium, Escherichia coli K 12 that did not metabolize NTA was affected only by concentrations above 5% NTA (50,000 mg/l), but between 0.5-5.0% altered cell morphology was noted. NTA exerted no mutagenic effects. Yellow pigmentation was noted when strains were grown on agar containing 50 mg/l NTA. Recombinant formation was inhibited, but only when cells were grown in NTA prior to mating (65).

Algae

Work has been done with a limited number of species in culture but studies of effects on algal communities over time are lacking. Christie (15) noted no toxicity to Chlorella pyrenoidosa even at 275 mg/l. Sturm and Payne (67) found the 96 hr. TL_{50} for Navicula seminulum to be 185 mg/l in water with a hardness of 60 mg/l $CaCO_3$ and 477 mg/l in water with 170 mg/l hardness. They also tested the effect of NTA on Selenastrum capricornutum in Algal Assay Procedure (AAP) bioassays. NTA at 5 mg/l had no biostimulatory effect when added alone or with sewage effluent to water from eight U.S. lakes including some with very soft water (6.34 mg/l $CaCO_3$, lowest reported hardness value). Slight inhibition was noted in one instance. Similar results were obtained by these workers with Anabaena flos-aquae and Microcystis aeruginosa. NTA addition slowed the rate of or prevented Microcystis die-off, probably by chelating metals present at toxic levels in some waters.

Others(22) investigated the effect of NTA on growth of Cyclotella nana in 72 hr. laboratory studies. In natural seawater, concentrations of 1.0, 2.5, and 5.0 mg/l NTA had an inhibitory effect on cell yield and ^{14}C bicarbonate incorporation but in synthetic seawater 20 mg/l NTA had no effect. The authors attribute this to the more abundant concentrations of trace metals in the synthetic medium and reiterate the finding of Provasoli (54) that a chelator: trace metal ratio exceeding 3:1 may cause a trace metal deficiency. The toxic effects of 50 µg/l copper added to natural seawater were nullified by the addition of 0.5 mg/l NTA.

NTA (10 mg/l) added to enriched seawater medium has been reported to stimulate growth of the red tide dinoflagellate, Gonyaulax tamerensis (84). Photosynthesis was also stimulated in several tests but to varying degrees. No effect was noted on the growth or photosynthesis of Phaeodactylum tricornutum or natural populations dominated by Skeletonema and Rhizosolenia, but testing was not as extensive. Martin (43) cautions that these observations were obtained under laboratory conditions and may not be applicable to the natural environment. In other studies (18), no stimulation of the Florida red tide organism Gymnodinium breve by NTA was observed although concentrations up to 10 mg/l were non-toxic.

Christie (15) noted that NTA could serve as the sole nitrogen source for *Chlorella* but was not as effective as equivalent amounts of NO_3-N, but others (28) have reported that NTA could not serve as a sole nitrogen source for *Ankistrodesmus falcatus* or *S. capricornutum*. These observations are pertinent to the suggestion made by some that nutrient enrichment of surface waters (in particular those in estuaries) with nitrogen from NTA may lead to nuisance growths. Estimates have been made that the use of 10% NTA in detergent formulations could conceivably increase the N and C content of sewage effluents by no more than 0.7 and 0.8 percent respectively (67) and that N concentrations in surface waters might be increased 0.35 percent (75). Sturm and Payne (67) and Forsberg and Wiberg (28) found no stimulation of *S. capricornutum* growth when either NO_3-N or bicarbonate-C were added alone at levels expected from the degradation of 5 mg/l NTA.

Invertebrates

Flannagan (24) tested the effects of Na_3NTA on seventeen species of macroinvertebrates (Chironomidae, Trichoptera, Ephemeroptera, Odonata, Amphipoda, and Gastropoda) and two amphibians (larval salamanders and leopard frog tadpoles) in 96 hr. bioassay tests. Results indicated that the compound was not directly toxic up to 500 mg/l in both soft (0-20 mg/l total dissolved solids) and hard waters (170 mg/l total dissolved solids). Where toxicity was observed, it was attributed to pH increases in unbuffered waters. The author points out, however, that sewage effluents tend to be buffered and the pH changes should be minimal. Arthur, *et al*. (5) also attributed acute toxicity of NTA to the amphipod *Gammarus pseudolimnaeus* Bousfield to increased pH. The chronic toxicity of NTA to *G. pseudolimnaeus* was also tested and a "no-effect" level" of 19 mg/l was reported. Biesinger, *et al*. (9) tested NTA and NTA-metal complexes for chronic toxicity to *Daphnia magna* in eight natural waters ranging in hardness from 27-438 mg/l $CaCO_3$. Lethality and reproductive impairment were evaluated over 21 days. As hardness increased, NTA toxicity decreased (strong negative correlation; *r* = 0.95). The three-week LC_{50} values (mg/l Na_3NTA) ranged from 100 - 900 in waters with total hardness of approximately 35-450 mg/l. Changes in pH were small (<1 pH unit) throughout the study and chronic toxicity was attributed to anionic NTA present in excess of the molar equivalence of metal ions, an unlikely environmental situation. Reproductive impairment (decrease in number of young born) was reported to be 16% at concentrations of 57-70% of the three-week LC_{50} concentrations for unammended and artificially hardened Lake Superior water. Fifty percent reproductive impairment occurred at concentrations of 71-84% of the three-week LC_{50} concentrations. It was also noted that the toxicity of zinc and copper ions was eliminated by chelation with NTA, confirming an earlier report (64).

Flannagan (25) studied the effect of Na_3NTA at concentrations ranging from 6.25 - 100 mg/l (in water of approximately 117 mg/l total dissolved solids) on four generations of the snail Helisoma trivolis. Growth (measured by weight increase) and fecundity of all animals including the controls decreased with each succeeding generation. There were no significant differences in growth between the control or populations exposed to 6.25 or 12.5 mg/l NTA. Concentrations of 25, 50, and 100 mg/l depressed growth and fecundity but even at 100 mg/l, NTA was not acutely toxic. The depression was most apparent in the first generation and was reduced in each succeeding generation. Similar studies with species known to be less adaptable to environmental change are needed.

Fish

The reader is referred to the review of Thom (76) for a critical review of some early studies. In general, little attention was given to the chemical speciation of NTA in these tests. Toxicity observed at high concentrations may reflect uncomplexed NTA. Sprague (64) observed that NTA could protect the brook trout Salvelinus fontinalis from the acute toxic effects of copper and zinc when tested in soft water (14 mg/l $CaCO_3$). He also noted that NTA at concentrations of 100 mg/l was toxic and attributed this to increased pH. Acute toxicity to the fathead minnow Pimephales promelas (96 hr. TL_{50} value 114 mg/l NTA) was also attributed to high pH (5).

Acute toxicity of NTA to bluegills (Lepomis macrochirus) was tested in static bioassays (67). The TL_{50} was 252 mg/l in water with hardness of 60 mg/l $CaCO_3$, and 487 mg/l in hard water (170 mg/l $CaCO_3$). Dynamic bioassays run in parallel yielded similar results. Additional static tests with bluegills in soft water (35 mg/l $CaCO_3$) provided 96 hr. TL_{50} values of 198 mg/l (175-225, 95% confidence interval). In flowing water bioassays with the fathead minnow (P. promelas) a 96 hr. TL_{50} of 127 mg/l (93-170) was obtained and with rainbow trout (Salmo gairdnerii) 98 mg/l NTA (72-133).

Macek and Sturm (41) reported the results of 28 day exposure of bluegills and fathead minnows to NTA concentrations ranging from 5.6 - 200 mg/l in continuous flow bioassays using reconstituted water of 25 mg/l $CaCO_3$ hardness. Only at 172 mg/l was mortality due to NTA observed. At 96.0 mg/l mortality was not greater than in controls. Examination of gill tissue after exposure to NTA indicated no direct histological change. When the gills of other fish transferred to clean water following exposure were examined, NTA apparently induced no predisposition to pathogenic conditions. In another study with P. promelas chronic toxicity was not observed at the highest exposure level (54 mg/l NTA) (5).

SECTION 4

METHODS AND PROCEDURES

NTA ANALYSIS

Colorimetric Analysis

The zinc-Zincon method of Thompson and Duthie (77) was used without change. The claimed limit of sensitivity for this method is 0.2 mg/l; significant loss of precision was observed below 0.5 mg/l.

Gas Chromatographic (GC) Analysis

1. General: Methods for processing the samples were similar to those of Aue, *et al.* (6). Unfiltered 50-ml samples of NTA in stream water were acidified, cleaned by anion exchange (Bio Rad AG 1-X2), eluted (10 ml of 16 *M* formic acid) into a screw-cap test tube, and evaporated to dryness in a Kontes tube concentrator (85°C).

Pure acids other than NTA, used in screening experiments designed to show that other commonly occurring acids would not interfere in the GC analysis, were weighed directly into a test tube. The pure acid (or the dry residue from evaporation of a water sample) was suspended in 3 *M* HCl in *n*-butanol (2.0 ml), heated at 85°C for 30-35 min. and stirred intermittently with a vortex mixer. The solution was then evaporated to dryness with a stream of dry air at room temperature. Just before analysis, dry acetone (0.80 ml) was added and solution was transferred to a dry sampling vial having a PTFE-faced seal. Dibutyl phthalate (10 µl of a 10% solution) was added to each vial as an internal standard.

2. Reagents: The disodium salt (monohydrate) of NTA was used in most analytical and ecosystem studies. It was purchased (Aldrich Chemical Company) as a specially purified, "99+%" material. Analysis of its tributyl ester by GC showed no detectable impurities. Further purification of NTA was achieved by repeated recrystallization of the free acid from 1:1 (v:v) dimethylformamide:water. Other carboxylic acids were of the highest available commercial quality. Acetone and *n*-butanol

were reagent grade, redistilled from $MgSO_4$ in an all-glass apparatus. Acetone was stored over 4A molecular sieves. Hydrogen chloride, generated by adding concentrated H_2SO_4 to solid NaCl, was dried by passage through H_2SO_4 in an oven-dry apparatus before dissolving in n-butanol. The HCl concentration was determined by titration with standard NaOH solution to a phenolphthalein endpoint. $(1\text{-}^{14}C)$ NTA was supplied by Dr. R. L. Downey (Procter and Gamble, Cincinnati, Ohio, U.S.A.) as a $10^{-3}M$ solution of the trisodium salt containing 3.7×10^6 dpm/ml. $(2\text{-}^{14}C)$ NTA was synthesized from glycine and $(2\text{-}^{14}C)$ bromoacetic acid and purified by anion exchange chromatography.

3. Gas Chromatographic Conditions: The gas flow rates to the flame detector were 300 ml/min. of air and 40 ml/min. of hydrogen. The carrier gas (helium) flow rate was 55 ml/min. Injection-port and flame-detector temperatures were held at 250°C. Temperature program I, which was used to separate all the acids as their n-butyl esters, was isothermal at 145°C for 8 min. and then increased 6°/min. to 240°C. Program II, used for rapid analysis of NTA, was isothermal at 185°C. The retention time of NTA was ca. 19 min. on program I and 9-10 min. on program II. The automatic sampler was set to inject 4.5 µl amounts of test solution.

CHELATION OF METAL IONS FROM SEDIMENTS

Sediment cores (7 cm depth) were taken from the White Clay and Red Clay Creeks and from near a small municipal sewage treatment plant also on the Red Clay. Duplicate cores were sterilized in screw-cap jars and incubated aerobically with varying (10^{-7} to 10^{-3} M) concentrations of sterile trisodium NTA, sodium citrate and disodium IDA solutions.

Concentrations of sediment-derived Mn, Fe, Zn and Cu in the overlying water were analysed by atomic absorption together with a control (no chelating agent added). The samples were removed aseptically with 10 ml syringes after 1, 15, 16 and 29 days. Between days 15 and 16 the sample bottles were swirled, disturbing the top of the sediment and exposing fresh material to the potential chelating activity of the solution.

On the 29th day, samples were removed for sterility testing and for NTA (citrate, IDA) analysis.

At the conclusion of the 29-day sterile period, each jar was recharged with fresh NTA solution having the same concentration as at the start of the experiment. At the same time, a small portion of non-sterile sediment was added to provide a microbiological inoculum. Analyses for Mn, Fe, Cu and Zn were done after 1, 5, 8 and 14 days. The jars were bubbled with air twice daily to ensure aerobic conditions.

CHLORINATION OF IDA

Disodium IDA monohydrate (1.00 g, 5.17 mmoles) was dissolved in 100 ml deionized water and 22.1 ml (15.5 mmoles) of Clorox (5.25% NaOCl) was added. A sample was taken immediately and run on TLC using 30/110/40 benzene/ethanol/water. Detection was by PAN spray (55) or 1% potassium iodide. The reaction mixture showed a major spot at R_f 0.78 (IDA $R_f = 0.62$).

Attempts were made to esterify portions of the sample with dimethyl sulfate, methyl iodide, diazomethane, benzyl chloride, and thionyl chloride under various conditions but no treatment showed major peaks other than IDA esters.

Column chromatography and preparative TLC led to extensive decomposition of the compound.

Precipitation of the crude reaction mixture from aqueous solution with acetone afforded a white solid containing mostly IDA. Attempts to recrystallize the material from ethanol led to decomposition.

In an experiment designed to show whether the compound was stable at various pH's, IDA disodium salt (250 mg, 1.28 mmoles) was dissolved in 0.2 M phosphate buffers (pH 2,7, or 12) and bubbled with chlorine for 2 min. followed by flushing for 15 min. with air. The reaction mixture was immediately treated with excess KI solution and the optical density at 570 nm read. The values were corrected for a blank with IDA absent. Then the flasks were stoppered and allowed to stand for 17 hr., when another reading was taken.

ALGAL EXPERIMENTS

Experiments were conducted in a laboratory greenhouse under natural solar radiation. Radiation records are available but were not used in analysis. The diatom dominated communities were developed on glass slides in test boxes similar to those described by Patrick (45). For the supply of water, each box was connected to a 19 liter reservoir. Duplicate experiments were run for each test concentration. The reservoir for each box on a test concentration was connected to a 100 liter reservoir. During seeding once thru water came directly from a header tank to the test boxes at a rate of approximately 760 l/hr in order to establish maximum diverse populations of diatoms. Previous experiments had shown that a flow in this range for these boxes was optimum for the development of diverse communities.

When microscopical examination determined that similarly diverse diatom communities were developed on all slides, the experiments were started. In order to greatly reduce any future seeding, water was filtered through cellulose fiber filters.

These filters also excluded the entrance of most particulate matter. The 100 liter reservoir was changed daily so that variability in the concentration of NTA in the test could be reduced. The turnover time in each of the 19 liter reservoirs was about four hours.

The pH and temperature were automatically recorded at intervals of about 15 minutes. For these tests water was automatically withdrawn from the 19 liter reservoir and passed over the probes. The pH probes were calibrated daily with buffer solution of known pH. The temperature probes were checked with a calibrated thermometer. The general chemical characteristics of the water during the tests were determined at various intervals, Table 1. Previous experiments using White Clay Creek water have shown these are the frequency of analyses necessary for maintaining a reliable record of the characteristics of the water. It was sometimes necessary to add small amounts of Mn, Fe, and PO_4, in order to maintain similar ambient concentrations in all tests. Mn was added as $MnSO_4 \cdot H_2O$, Fe as $FeCl_3 \cdot H_2O$ and phosphate as NaH_2PO_4. The NTA was added as disodium NTA and was determined one or two times a day.

In these experiments it was found that a fairly large predator pressure might develop on the algal communities. This predator pressure was mainly that of aquatic insect larvae. For these reasons, the slides were carefully examined under a microscope several times weekly and herbivores removed. It was, of course, impossible to remove the protozoan predators.

During the course of the experiment frequent microscopical observations were made as to diversity of the algal communities, species dominance, and condition of the algal cells. Concentrations of chlorophyll a, chlorophyll c and phycocyanin pigments; and biomass were determined at the beginning, during and at the end of each experiment. Primary production measured by ^{14}C uptake, and the uptake of various metallic ions was measured three times during the experiments. The relative dominance of the more common species of diatoms was determined by cell counts.

Primary Productivity- ^{14}C Uptake

A single slide was selected from each test unit for determining primary productivity. The community on one side was removed and placed in a Petri dish with 35 ml of water from the test system for dark incubation. The community still intact on the other side was placed in a second Petri dish with 35 ml water from the system for light incubation. Isotope ($NaH^{14}CO_3$, New England Nuclear, Boston, MA, sp. act. 8.4 mCi/mM) was added to each dish to provide a final concentration of 1 μCi in 35 ml. The sample for dark incubation was covered immediately with aluminum foil. Incubation was conducted for 1 to 2 hours at naturally occurring temperature under fluorescent lights with a spectrum closely approximating solar radiation ("Vita lite", Duro Test Corporation, North Bergen, NJ). Incorporation was terminated by adding 1 ml of 37% formaldehyde.

A sample (10 ml) of the water from the sample incubated in the light was taken, membrane filtered (0.45 μ pore size, Millipore Corporation, Bedford, MA, at 0.5 atm), acidified with 0.5 ml 0.5 *N* HCl to pH 3.0, bubbled for 30 minutes to drive off unincorporated bicarbonate, and neutralized with 0.5 ml 0.5 *N* NaOH. The ^{14}C remaining in the water as organic material excreted by the algae was determined by liquid scintillation counting of a 3 ml subsample in a toluene-Omnifluor (New England Nuclear, Boston, MA) - Triton X (Beckman Instruments, Fullerton, CA) cocktail. Water samples were counted with 89% efficiency.

The biomass was scraped from the slide used for light incubation. The algae and water for each light and dark incubation were transferred to individual 50 ml plastic test tubes and centrifuged at 12,000 x gravity for 10 min. at 4°C. The pellet was resuspended in 0.1 *M* phosphate buffer (pH 7.0) and brought to a final volume of 5 ml after which it was homogenized briefly with a teflon homogenizing pestle driven by a stirring motor. Five 0.25 ml amounts were removed, membrane filtered at 0.5 atmospheric pressure, and washed with successive rinses of distilled water. The filters were air dried and exposed to fumes of concentrated HCl to remove any adsorbed radioactivity. Incorporated radioactivity was determined by liquid scintillation counting after combusting the samples in a sample oxidizer (Packard Instruments, Naperville, IL) and collecting the $^{14}CO_2$ from combustion as a carbamate compound. These samples were counted with 62% efficiency.

Five 0.5 ml aliquots from each homogenized community were transferred to test tubes and 7.0 ml acetone (made basic with the addition of a pinch of $CaCO_3$) was added. The chlorophyll *a* content was determined after overnight extraction at refrigerated temperatures. The samples were centrifuged to pelletize the algal cells and the chlorophyll *a* was determined from optical density readings before and after acidification to correct for phaeophytin content (40).

The values for the five replicate chlorophyll *a* determinations for each light or dark incubation were averaged and reported as μg chlorophyll *a* for the algae on one side of slide. Radioactivity determinations for each sample type were corrected for counting efficiency, averaged and the incorporated radioactivity value was normalized for chlorophyll *a* concentration. Values reported have been corrected for dark adsorption and excretion.

Chlorophyll a and c Extraction and Analysis

Extraction (60). The algae on one-third of one side of each slide were collected and excess water removed.

The biomass was extracted with 4.0 ml of 80% dimethylsuflox-ide (DMSO) in 25 or 50 ml flasks with shaking on a wrist-action shaker. The yellowish extract was filtered through Whatman No. 1 paper into side-arm test tubes.

The filtrate contained most of the chlorophyll c (in this work, specific for diatoms) and some chlorophyll a. The residue on the paper was extracted with 4.0 ml acetone (made basic with $MgCO_3$) by shaking for fifteen minutes in stoppered flasks. The samples were filtered as before and the green extract was reserved in stoppered tubes for analysis of chlorophyll a.

Analysis. The spectrum of each extract was determined from 550 - 750 nm using a Beckman DBGT recording spectrophotometer (Beckman Instruments, Fullerton, CA). Chlorophyll c absorbance was determined at 570 and 630 nm and chlorophyll a absorbance at 665 nm. Concentrations were computed from published equations (60).

The a/c ratio was determined by adding the two concentrations of chlorophyll a in the two extracts and dividing by the chlorophyll c concentration in the DMSO extract. A low ratio (8 or less) indicates a high proportion of diatoms; pure cultures of marine diatoms average a ratio of 4 (33).

Phycocyanin Extraction and Analysis

Extraction. The algal community was scraped from one side of a slide as before. Excess water was removed and the cells were suspended in 8 ml of 0.005 M pH 6 phosphate buffer. The suspension was sonicated in a stirred ice bath in the dark for 12 minutes, and centrifuged at 12,000 rpm for 15 minutes.

Column Chromatography. The columns were prepared by filling 25 ml burets with Bio-Gel HT hydroxyapatite (added from a well-shaken suspension) until the column was about 40 cm high. The column was rinsed with 10 ml of 0.005 M phosphate buffer and the sonicate was added using a Pasteur pipet to minimize disturbing the top of the column. The sonicate was allowed to run through, using a slight vacuum; the solution was discarded. Pigments were eluted into a new collection vessel with 0.25 M phosphate buffer. The column was run until 7 to 9 ml of concentrated buffer had been collected or until all visible blue color was completely removed. The spectrum was run on this fraction (68).

Analysis. The absorption spectrum was determined from 750-500 nm. The phycocyanin peak appeared at 620 nm. The concentration of phycocyanin in mg/l was determined using the equation:

$$\text{Phycocyanin}_{\text{ppm}} = \text{O.D.}_{620} \times 1000/\text{Ext. Coeff.}$$

The extinction coefficient (7.74) reported by Troxler and Lester, (80) was used. O.D._{620} values were corrected for turbidity (O.D._{750}).

Biomass Determinations

The growth from one side of a slide was carefully scraped and placed in a crucible weighed according to quantitative analytical procedures. It was then dried to constant weight at 103°C and the weight was determined. The material was ashed at 600°C, dried to constant weight, and the ash free dry weight determined.

Extraction and Analysis of Metals

The extraction and atomic absorption analysis (Model 303 spectrophotometer, Perkin Elmer, Fort Washington, PA) of metals associated with the algal biomass was carried out according to the methods described by Allan (2).

BACTERIAL EXPERIMENTS

NTA Degradation: Flask Bioassays

Experiments were done with populations developed on coverslips held in Plexiglas microcosms. The microcosms held 100 coverslips and were placed in a water jacket through which stream water was passed once in order to maintain the populations at near ambient temperature. White Clay Creek water was passed through each microcosm at a rate of approximately 13.8 l/min. The microcosms were covered with foil to prevent the entry of light and thereby reduce algal development on the coverslips. The microcosms are illustrated and described more fully in reference 11. After the coverslips were seeded with the periphytic populations, the microcosms were converted to recirculating systems as described for algal studies.

Water was recycled through the boxes at a rate of approximately 760 l/hr. NTA was added as the disodium salt to maintain desired concentrations of approximately 0.02, 0.2, 2.0 and 20.0 mg/l. Prior to NTA exposure and periodically thereafter, coverslips with the attached microbial community were removed from each microcosm for use in studies of NTA degradation.

Measurement of degradation. The evolution of $^{14}CO_2$ from 1-^{14}C-NTA was monitored as a measure of degradation activity. After removing any attached larval forms, three coverslips were transferred to each of six biometer flasks (52) containing 10 ml membrane filter sterilized (0.45 µm pore size) stream water. Five replicate flasks were used in each mineralization assay. The sixth flask was a control with cells killed by the injection of Lugol's iodine prior to the introduction of ^{14}C NTA. Analysis of data from fifteen degradation rate assays showed that five replicates provided a number ± 30% of the mean with 95% confidence in thirteen instances.

Incubations were started by adding 0.5 µCi ^{14}C NTA (Amersham-Searle, Arlington Heights, IL; sp. act. 52.5 mCi/mM) to the water after which the flasks were stoppered to close the system. Degradation (evolution of $^{14}CO_2$) was measured at 0.2 mg/l for the populations exposed to approximately 0.02 and 0.2 mg/l in the microcosms. This was necessitated by the specific activity of the isotope. It was not possible to use a lower concentration of isotope in the assay system and insure that the isotope concentration would not be limiting. However, unlabeled NTA was added to biometer flasks to establish the desired concentrations for the organisms that were exposed to approximately 2.0 and 20 mg/l in the microcosms. Additions to the assay system were made from frozen stock solutions or from serial dilutions of the same. The NTA concentration in each bioassay flask was not determined but the concentration of 10X stock solutions were checked prior to several assays of degradation rate. Four determinations on the stock used for the 20 mg/l bioassay indicated concentrations of 255, 251, 258 and 263 mg/l. The stock used to establish concentrations approximating 0.02 and 2.0 mg/l in the bioassay flask contained 23.8, 19.3, 24.4 and 26.4 mg/l.

Incubations were conducted in the dark with agitation in a rotary water bath shaker at a temperature in the natural range for that time of year. The flasks have a side arm to which 0.5 ml of a saturated solution of KOH in anhydrous ethanol were added. Respired $^{14}CO_2$ from the labeled NTA was trapped in the solution. Every 16 min. for 64 min. the KOH was removed through the stopper for assay of radioactivity and replaced with fresh solution. The side arm was rinsed twice with 1 ml of anhydrous ethanol and the washings were counted with the 0.5 ml initially removed in Cocktail T (Beckman Instruction Manual 8.555-E, pgs. 2-11, Beckman Instruments, 1967) containing 2.5 percent Cab-o-sil. Samples were counted in a liquid scintillation counter at 89% efficiency.

Data from the linear phase of NTA metabolism were used to calculate the rate of breakdown expressed as ng NTA degraded/hr. The decomposition rate for the population on the coverslips was calculated as follows:

1) $\frac{\text{DPM mineralized}}{\text{DPM added}}$ = Percent mineralized

2) (Percent mineralized) x (ng NTA in assay system)

x (k_1) x (k_2) = ng/hr. mineralized/cm^2

k_1 = factor to convert rate to hourly rate

k_2 = factor to correct for surface area used

Estimation of microbial numbers. At the end of the incubation, the coverslips and the incubation medium from each flask were aseptically transferred to a grinding vessel and homogenized at <4°C. Serial dilutions were prepared and enumeration was done by the method of Harris and Sommers (32). NTA-agar was that described by Tiedje, et al. (79) with NTA as the sole carbon and nitrogen source. Plates were incubated at or near the temperature of assay and were scored at weekly intervals.

NTA Degradation: System Experiments

In some experiments, concentrations of the nitrogen end product from complete NTA degradation, ammonia, and its oxidation products nitrite and nitrate were measured in the recycle microcosm system. Ammonia-N was determined by the phenol-hypochlorite method, NO_2-N by the sulfanilic acid-α-naphthylamine hydrochloride procedure, and NO_3-N by the chromotropic acid procedure (4).

In another experiment, 8 µCi 1-^{14}C was added to a system whose volume was 87 l. Each hour, 2.9 l were replaced; thus, a predictable loss of ^{14}C NTA would result from dilution alone. The variance of the actual ^{14}C concentration from the predicted concentration would be indicative of the mineralization of NTA in the system.

Periodically, samples (25 ml) were collected and placed in Erlenmeyer flasks. The flasks were sealed with serum stoppers and the samples were acidified to pH 1-2 by the addition of concentrated HCl using a hypodermic needle and syringe. A cotton swab held in a cup suspended from the serum stopper was saturated with alcoholic KOH to trap carbon dioxide. The samples were agitated gently to elaborate $^{14}CO_2$ that resulted from the mineralization of the labeled NTA from the aqueous phase. Subsamples of the water were taken and the radioactivity associated with the CO_2 traps and remaining in the water was determined by liquid scintillation counting. Water samples were filtered through 0.45 µ membrane filters, but there was little difference in radioactivity associated with filtered and unfiltered water samples. Only background levels were associated with the filters.

Effects of NTA-Metal Chelates on Bacterial Activity

NTA-metal chelates were prepared by mixing NTA and metal salts in a 3:2 molar ratio in distilled deionized water. In all assays, thin-layer chromatograms (benzene/ethanol/water 30:110:40 as solvent, PAN developed) (55) were used to demonstrate that the metal ions were NTA chelated. Metals were added as the chloride salt. Chelates were usually prepared from the chloride salt except for lead - the lead acetate salt was used.

To avoid difficulties with varying cell inoculum on coverslips, cell suspensions were prepared by growing stream organisms in 0.1% yeast extract-tryptone broth overnight at the temperature of assay. The inoculum was loose silt or sand from an ecosystem stream. Some experiments were also done with NTA degrading organisms; a *Pseudomonas* strain (T-1) supplied by Dr. James Tiedje, Michigan State University, and isolate "216" from the White Clay Creek. These cells were cultured in NTA broth.

Cells were harvested and washed two times with sterile distilled water and resuspended in membrane filtered (0.45 μ pore size) White Clay Creek water. Cell numbers were determined by the plate dilution frequency assay (32). Cells (1 ml) were transferred to biometer flasks containing 22.75 ml membrane filtered stream water. Each flask received 0.25 ml of either NTA, metal salt, or NTA-metal chelate; metal ions were present in the assay flask at 1 mg/l, NTA at 4 mg/l. After a 16 min. pre-incubation, 1.0 ml ^{14}C-glucose (uniformly labeled, sp. act. 234 mCi/mM, New England Nuclear, Boston, MA) was added to give a final concentration of 1.0 μCi/flask. Incorporation was shown to be a more sensitive indicator of toxicity than mineralization. Therefore, cells were collected at 16 min. intervals for 64 min. and filtered onto membranes (0.45 μm) for determination of incorporated radioactivity by liquid scintillation spectrophotometry.

Another experimental approach was used to study the effect on bacterial activity of longer exposure (22 hr.) to metals or their NTA-chelates. Cells from an inoculum of mixed periphytic bacteria were grown overnight in 0.1% yeast extract, 0.1% glucose broth, collected, held in double distilled water for 2 hr. to deplete cellular reserves, and resuspended in broth to a final density of 8 x 10^6 cells/ml determined by direct microscopic counts. The respiration rate of 50 ml resuspended cells in 50 ml Erlenmeyer flasks was determined using a model 5331 dissolved oxygen probe and meter (Yellow Springs Instrument Company, Yellow Springs, OH) connected to a recorder (Microcord Model 44, Photovolt Company). Cultures were stirred continuously while these measures were made. Additions of metal, NTA, or metal chelates were made to establish desired concentrations, and respiration measurements were made immediately after addition, 2.5 hr. and approximately 22 hr. later. At the end of some experiments, a final respiration rate was measured after the addition of nutrients which stimulated respiration rate. At 22 hr. cell numbers were determined, and concentrations of test metal in the supernatant and associated with cells were determined. Metal concentrations were determined by atomic absorption spectrophotometry. Chelation of metal was determined by thin-layer chromatography and showed NTA-metal chelates were stable through the experiments. However, metals added alone chelated to some degree with organics in the medium during the experiment. Metals were used at a high concentration (100 mg/l) to arrest respiration rapidly (15 sec.) although one experiment was performed with 1 mg/l metal. Studies with Cu and Hg showed 15 min. were required to terminate respiratory activity when 1 mg/l was used.

EFFECTS AND FATE OF NTA AND COPPER IN ECOSYSTEM STREAMS

This experiment was performed to determine the activity and fate of NTA and copper when introduced into simulated natural environments (ecosystem streams) over long time periods either singly or together. Particular emphasis was placed on the mobilization and uptake of copper (and other selected metals) in the presence and absence of NTA.

Four 12 m long experimental stream systems with two riffles, slack water, and shallow pools containing natural substrates (silt, sand, gravel, and rocks) were seeded with natural communities for over two years prior to initiating experiments. Water from the parent stream (White Clay Creek) was passed once through each stream. Flow rate averaged 169 l/min. (c.v. between streams was 3.2%) and current velocities in riffle sections measured 30-42 cm/sec. Each stream was populated by diverse communities of diatoms and other algae such as Oedogonium, Vaucheria, and Spirogyra had seasonal population expressions. Rooted aquatics (e.g. Anacharis), and floating duckweed (Lemna sp.) were common in pool areas. A diverse invertebrate fauna with species of caddisflies, mayflies, worms, and snails populated each stream. Although population sizes varied between streams, representatives of each common species were found in each stream. The banks of each stream built up naturally with sediment and supported a thick growth of herbaceous plants of similar species composition. Additional description is provided in reference 11.

One stream was designated the control, one received NTA to maintain a concentration approximating 2.0 mg/l, one received 30 ug/l copper as $CuSO_4$, and the last received both NTA and copper at the indicated concentrations. NTA and $CuSO_4$ were metered continuously from concentrated stock solutions (using syringe pumps, B. B. L., Baltimore, MD) into the header tanks of each stream where rapid mixing occurred. Initially, water was not recycled, but 9.5 weeks into the experiment a 50 percent recycle was used.

NTA and copper concentrations in the water were monitored daily using the zinc-Zincon method and atomic absorption spectrophotometry respectively. NTA concentrations were also confirmed by gas chromatographic detection. Concentrations of NH_4-N, NO_2-N, NO_3-N, and PO_4-P were determined daily and other chemical parameters (e.g. total alkalinity, sulfate, chloride, silicate) were measured one-three times weekly (depending on expected concentration change) using Standard Methods (4) or other appropriate procedures. Concentration of several metals other than copper (Ca, Mg, Fe, Mn, Zn) were measured several times weekly by atomic absorption spectrophotometry.

Prior to the addition of Cu^{++} and/or NTA and at weekly intervals thereafter samples of sediment were taken for metal analyses and less frequently for NTA analysis. Five cores were taken from each stream at each collection time. Sediment samples (primarily silt) were collected by inserting a parafilm coated metal tube (10 cm diameter) into the sediment and filling the tube with dry ice and ethanol. The probing device had a plastic funnel tip to facilitate entry into the sediment. The dry ice froze the sediment for 3-4 cm around the core, and the core with adhering frozen sediment was removed. Samples of frozen sediment were removed at selected depths (surface, 2 cm, and 5 cm) by cutting away from the frozen material. The sediments were dried, sieved, and the fraction $\leq$ 1 mm was saved for analysis of both exchangeable heavy metals. Preliminary studies demonstrated higher metal content in this size fraction compared to larger size fractions. Levels of exchangeable Cu, Fe, Mn, Mg and Zn were determined by atomic absorption spectrophotometry after extracting ten grams of sediment with 20 ml of DTPA (Dimethylenetetramine pentaacetic acid) (39).

This method of sediment sampling led to mixing of the unsampled material because sediment from adjacent areas collapsed into the hole created when the frozen core was removed. Only a small number of samples could be taken from the limited area of each pool before this mixing appeared to alter concentration data at different depths.

Another approach was used to evaluate the potential sorption or release of metals to or from these sediments resulting from NTA exposure. Containers (10 cm w x 10 cm l x 12 cm d) were filled with sediments from the parent stream that were mixed to make them relatively homogenous and put in 22 l microcosms containing stream water (control), 2 mg/l NTA + 30 μg/l Cu^{++}, or 30 μg/l Cu^{++} in stream water. The solutions were recycled over the sediments in the microcosms and the systems were held at environmentally realistic temperatures in a water jacket through which stream water was passed continuously. Solutions were changed daily and concentrations were monitored daily (Cu^{++} by atomic absorption spectrophotometry; NTA by zinc-Zincon analysis (with occasional gas chromatographic confirmation). Containers of sediment were removed at intervals and frozen. Samples of the upper cm, 2 cm, and 5 cm depths were taken by cutting through the frozen block. The perimeter of each layer was removed and the central area was used for DTPA extraction and a.a. metal analyses.

In order to determine possible accumulation or depletion of metals in the biota, samples of algal communities, aquatic vascular plants and animals were analyzed for concentrations of Cu, Fe, Zn, Mn, and Mg. Mixed communities of periphytic algae dominated by *Melosira varians* (sample size; 30-600 mg dry weight) *Lemna*, and *Anacharis* (sample size; 40-90 mg dry weight, 20-50 mg dry weight, respectively) were sampled at two locations in each stream. *Anacharis* samples were taken at the growing tips of the plants. *Tubificid* worms (sediment feeders) were collected from sediments in pool areas and *Planaria* sp. from the surfaces of sediments and plants in the *pools* (sample size; 10-40 mg dry weight for both organisms). Significant differences between sampling locations within a stream were not obtained.

Samples were dried at 100°C for 24 hr. and desiccated overnight at room temperature. After weights were taken, the samples were combusted at 500°C for 2 hr. and organic weights obtained.

Ashed plant material was extracted twice in 20% HCl with warming. The extracts were pooled and diluted to 50 ml with a 1% lanthanum oxide solution as a releasing agent to allow for Mg determination. Metal concentrations were determined by atomic absorption spectrophotometry.

Ashed animal material was extracted by adding 1 ml of concentrated HNO_3 and 2 ml of deionized water to the crucible. The extract was brought to a boil and after settling, the extract was aspirated off and its volume adjusted to 5 ml with a 2.5% solution of lanthanum oxide. Concentrations of the same five metals were again determined and reported as for the plant material.

SECTION 5

RESULTS AND DISCUSSION

CHEMICAL STUDIES

Analysis of NTA

Gas chromatography. (38) One of the prime goals of this study was to develop a method of NTA analysis which was reliable, reproducible, and free from inorganic and organic interferences at low NTA concentrations. Because we also wished to be able to look for possible degradation products of NTA, we focused our attention on gas chromatographic (GC) methods, particularly that of Aue, et al. (6) because it was demonstrated that NTA could be freed from chelates and some organic interferences by ion exchange cleanup. In addition, analyses by this method were very good down to well below 15 μg/l (our lower study limit), and good recovery of added NTA from very hard tap water, and also from sewage effluent, was shown.

There have been no thorough studies of the interference of organic substances in NTA analysis. Taylor, et al. (73) showed that the trimethylsilyl ester of NTA eluted between those of lauric and myristic acids, but did not report studies with other interfering substances. Citric acid tributyl ester has been reported to co-elute with the tributyl ester of NTA on Carbowax 20 M columns. The Aue GC method has been particularly successful in affording rapid reproducible analysis at concentrations in the μg/l range. In particular, citric acid is well resolved from NTA. However, it requires the use of a special column consisting of Carbowax 20 M on acid-washed Chromosorb W. The column must first be strongly heated and then continuously extracted for prolonged periods, leaving a thin film of unextractable polymer on the support (7).

The difficulties in this approach prompted us to investigate the capabilities of more common, commercially available, GC liquid phases, especially those similar to phases already used for analysis of esters. After some preliminary screening experiments, we found excellent resolution of NTA tributyl ester and the structurally similar citric acid ester on a commercial 3% OV-210 column. The two compounds were separated by approximately one minute (depending on the individual column) when an isothermal (185°C) run of 10-15 minutes was used. Next, we attempted to find conditions for the analysis which would separate NTA from some of its possible degradation products and also from some potentially interfering, naturally occurring acids.

Initially, weighed amounts of each of thirty-six pure acids were separately derivatized and injected into the gas chromatograph. Eleven acids (oxalic, capric, caproic, caprylic, lauric, oleic, protocatechuic, salicylic, chlorogenic, caffeic and gallic) gave no detectable peaks and were not further studied. Peak areas for the remaining twenty-five acids were electronically integrated and their molar responses were determined relative to dibutyl phthalate. Retention times were also corrected to that of dibutyl phthalate. Table 2 summarizes this data.

An equimolar mixture of these acids was dissolved in aqueous acetone. An aliquot of this mixture, containing 2 umoles of each acid, was evaporated to dryness, derivatized and analyzed by temperature program I (145°C for 8 min, then 6°/min to 240°C). Fig. 4 shows the resulting GC trace; the NTA tributyl ester peak appeared between those of the butyl esters of arachidic and sinapic acids. Separation from citric acid tributyl ester was excellent (ca. 1.0 min) using this program, and also (ca. 0.8 min) using the isothermal program II. Although separation from arachidic acid butyl ester was incomplete using program II, this program was adopted for routine analysis of NTA in stream water. Ion exchange completely separated the two acids, and, furthermore, it seemed unlikely that detectable amounts of arachidic acid would occur in our stream water.

The analysis of NTA was next studied in detail. First, a standard curve was constructed from measurements on samples of water from White Clay Creek to which NTA had been added, giving concentrations ranging from 0.01 to 200 mg NTA per liter. Samples (50 ml) were subjected to an ion-exchange and derivatization procedure (cf. Methods and Procedures Section 3) and injected in triplicate into the 3% OV-210 column. Fig. 5 shows the graph of integrated area vs. NTA concentration. It was possible using the procedure and a well-conditioned column to analyze NTA reliably at 10-25 μg/l. As the columns aged, the NTA peak broadened and eventually became undetectable. The average lifetime of a newly conditioned column was on the order of 200 injections.

Recovery of NTA by the procedure was studied by use of (^{14}C)-NTA. Five replicate samples of a 0.20 mg/l solution were taken through the ion-exchange and derivatization steps and examined periodically for radioactivity. The results (Table 3) showed that 98% of the starting NTA adhered to the ion-exchange column, 86% of the total was recovered after elution with 16 M formic acid, and 75% remained after conversion into the n-butyl ester. Thin-layer chromatography (silica gel/diethyl ether) on the radioactive esterification product showed a single spot containing 95% of the total activity; the remainder was mainly at the origin.

The data demonstrates that NTA can be recovered, its tributyl ester formed in high yield, and specifically detected by gas chromatography, starting with a dilute solution of NTA in stream water.

In screening experiments, the OV-210 column was superior to columns packed with equivalent amounts of OV-1 or OV-225. On OV-1, separation from citric acid butyl ester was good, but the NTA peak was close to those of the syringic and plamitic acid esters. On OV-225, the NTA and citric acid ester peaks were close together. We repeated the separation on different OV-210 columns from Applied Science Labs., obtained at different times, with practically identical results.

A few acids were not resolved by program I. Linolenic, linoleic and stearic acid ester peaks overlapped almost completely and several others were not resolved to baseline levels.

However, NTA and its likely degradation products, IDA and N-methyl-imino-diacetic acid (MIDA, Fig. 1) were well separated from many potentially interfering acids; only arachidic acid, a relatively uncommon fatty acid, could have caused difficulties if present in large excess.

A disadvantage of the GC method is its slowness; cleanup, derivatization, and analysis of 4-12 samples requires over six hours. In addition, it does not distinguish between "free" and "complexed" NTA. However, we believe that the potential of the method for the analysis of complex natural mixtures, and its freedom from interferences, may make it applicable in a wide range of situations.

Colorimetry. In samples containing higher (over 0.2 ppm) concentrations of NTA, we tested other procedures based on colorimetric measurements of total complexing ability in order to assess more rapid methods of NTA determination. The zinc-Zincon procedure is a standard colorimetric method for the analysis of NTA in detergent formulations and waste waters. The authors claim sensitivity down to 0.2 ppm NTA (71).

In our hands, the procedure seemed quite reliable at concentrations of NTA exceeding 2 ppm. Below 2 ppm, there was increased scatter, and the method was not highly accurate below 0.5 ppm.

A major drawback of the method is its susceptibility to interference from other metal-complexing agents. For example, we showed that IDA, a known metabolic product of NTA is nearly as good a complexing agent as is NTA; on a molar basis, IDA maintains copper in solution 70% as well as does NTA. Even glycine, another possible NTA breakdown product, has significant complexing ability for copper. It is conceivable that under some environmental conditions, these products or other significant complexing agents could accumulate in NTA-containing systems. Therefore, a method of NTA analysis based on total metal-chelating capacity could conceivably afford erroneous results.

However, in experiments in biological systems in which NTA analysis was done using GC and the colorimetric method, we did not encounter serious discrepancies. Below 2 ppm, the zinc-Zincon method appeared on the average to give somewhat higher values than did GC. Regression analysis for 503 samples in which NTA was determined by both procedures indicated a high degree ($r = 0.962$) of correlation between them. The equation of the line was: mg/l NTA (zinc-Zincon) = 1.036 x mg/l NTA (GC) + 0.356.

Chelation of Metal Ions

In natural waters, NTA will exist in chelated forms almost exclusively. A computer simulation for equilibrium conditions was carried out (by J. Chance, as part of a M.S. thesis at the University of Pennsylvania) using published data for NTA chelate stability, concentrations of metal ions in White Clay Creek, and a 10^{-6} M NTA concentration. Most (85%) of the NTA was predicted to occur as a mixture of two iron-containing forms, Fe (OH) NTA and Fe $(OH)_2$ NTA. Possible contributions by colloidal oxides of iron were not, however, taken into consideration. The copper complex made up 8% of the total NTA; almost all of the remainder was present as the lead (6%) and nickel (1%) chelates. The results differ from those of Childs, who showed that in Lake Ontario, copper-NTA would predominate at equilibrium (14). The difference largely reflects the low abundance of copper in White Clay Creek.

Computer and spectroscopic studies on the displacement of metal ions in NTA chelates by free cupric ion (Chance, unpublished M.S. thesis, University of Pennsylvania) were done. Reactions between Cu^{++} and NTA complexed with Mg, Ca, Zn, Cd and Na were too fast (<1 sec) to measure spectroscopically. Fast but measurable rates were obtained using the Ni, Pb, Fe, and Co complexes. Detailed study of the NTA-Co + Cu^{++} reaction showed a rate constant of 35.7/mole/sec.; the data were consistent with an S_n2 displacement mechanism. The reaction was observed to reach 90% completion within 75 sec. The evidence indicates that attainment of equilibrium among NTA complexes in a natural water system would be a fast process relative to their decomposition by biological or chemical means. An approximate upper limit of four hours for the equilibration of the system was calculated.

Studies of the extraction of metal ions from sediments using NTA solutions were undertaken. Stream sediments from three locations (a relatively unperturbed area of White Clay Creek, Chester Co., PA; an area on Red Clay Creek, Chester Co., PA, immediately impacted by agricultural use; and another site on the same stream immediately below a small municipal sewage plant) were sterilized in jars containing NTA, citrate, and IDA in concentrations ranging from 10^{-7} to 10^{-3} M. The jars were allowed to stand for 29 days, then reopened and incubated with a small portion of non-sterile sediment from White Clay Creek. Figs. 6-9 summarize the data on metal ion concentrations.

In general, the results of the aseptic studies indicate that NTA did not lead to statistically significant* increases in concentration of the metal ions investigated at 10^{-5} M (1.9 mg/l) or less. In only one case (Red Clay sewage sediments and zinc) was there a significant increase at 10^{-5} M.

* $P \leq 0.05$ using Student's T-test

At 10^{-3} M (191 mg/l) there were significant increases in concentration of all metal ions tested ranging from 3 x (Mn, White Clay) to 15 x (Zn, Red Clay). The increases were not surprising; the NTA concentration approximated that expected in a household washing machine using a detergent containing 6% NTA, and was accordingly not considered to be environmentally relevant.

Agitation of the sediments at the midpoint of the study generally led to a slight increase in metal concentrations for all samples, including the control.

At the 10^{-3} M level, IDA (a known degradation product of NTA) increased the concentrations of all four metal ions; these increases were significant for iron, zinc, and (in one sediment) copper. Trisodium citrate (at 10^{-3} M), a natural metabolite stereochemically similar to NTA, significantly increased the concentration only of iron. For both IDA and citrate, significant increases in metal ion concentration were not observed at 10^{-5} M or less.

The results are in general agreement with those of previous studies (8,13,72) indicating no large increases in metal ion mobilization from sediments at NTA concentrations of less than 10^{-4} M.

The gradual decline in concentration of solubilized iron and manganese, especially noticeable at the 10^{-3} M NTA level, over the course of the sterile portion of the experiment was attributed at least partly to photooxidation (37,66). Additional support for the hypothesis is the finding that NTA showed decreases in concentration over the study period by amounts ranging from 36 to 68%. Test for sterility (by inoculating nutrient agar plates with overlying water from all experimental flasks) showed no bacterial or fungal contamination at the end of the 29-day period. Soluble zinc remained essentially constant, but soluble copper increased three fold (0.04 to 0.12 mg l^{-1} at 10^{-3} M NTA) during the sterile period. Chau and Shiomi (13) have shown that, at 2 ppm in lake water, the NTA complex of copper is resistant to biological breakdown. The present results may indicate a similar chemical unreactivity or gradual release of strongly chelated copper from the sediments.

In all the non-sterile sediments exposed to 10^{-3} M NTA, there was a sharp drop in copper concentration. The drop was especially marked with the sewage plant inoculum; within a day the copper concentration was below 0.01 ppm and indistinguishable from control samples.

Manganese concentrations in the 10^{-3} M NTA experiments became relatively stable after an initial surge. In the White Clay and sewage plant samples, the concentrations dropped to

7-8 ppm (approximately what they had been under sterile conditions); but the Red Clay samples dropped to background levels (<0.5 ppm).

Iron concentrations tripled in each 10^{-3} $\underline{M}$ NTA sample over the first 8-day period. A concomitant increase in sheathed iron-utilizing bacteria was observed microscopically. However, by the 14th day iron concentrations had dropped back to their initial levels, except in the case of the sewage plant sediments where they remained high. Iron concentration in the sewage controls also increased, however.

Zinc in the 10^{-3} $\underline{M}$ NTA samples dropped gradually over the 14-day non-sterile period. In the case of the White Clay sediments, but not in the other samples, there was an initial surge in concentration from 0.5 to 1.25 ppm.

In an attempt to determine the extent of solubilization of the metal ions, we analyzed all the sediments for metal content. An arbitrary assumption was made that the metal ions in the top centimeter would be available for extraction by NTA.

The resulting data for the 10^{-3} $\underline{M}$ NTA experiments is summarized in Table 4.

About 5% of the "available" copper was brought into solution at the White Clay and upstream Red Clay sites. A somewhat higher (8.5%) degree of solubilization was observed at the sewage plant site; the copper content of these sediments was much higher.

Results quite similar to those for copper were observed in the zinc analyses. The sewage plant sediments likewise were considerably enriched in zinc and a relatively larger percentage of zinc was solubilized. It is possible that some of the zinc and copper at the sewage plant site was in forms more easily accessible to NTA complexation.

At all sites, about 3-5% of the "available" manganese was solubilized.

In general, iron was not solubilized to a significant extent; much less than 1% of the "available" iron was chelated in all cases. Presumably this reflects the existence of iron in strongly chelated, otherwise highly insoluble, or oxidized form not available to NTA chelation.

Chlorination of NTA and Derivatives

Some chlorinated derivatives of hydrocarbons are among the most toxic of known synthetic compounds. Accordingly, chlorinated derivatives of NTA or of its degradation products might have a greater impact upon ecosystems than the parent compounds. Such chlorination could occur in sewage treatment processes; recent investigations have shown that reactive organics, such as phenols, are the precursors of a wide variety of chlorinated compounds (34).

Tertiary amines, such as NTA, can be chlorinated by drastic, oxidative processes leading to N-chloro compounds and aldehydes (19). More likely is the direct chlorination of a secondary amine such as IDA. This compound might accumulate in some environmental situations, since some NTA-degrading bacteria do not metabolize IDA (17).

In preliminary studies designed to test these possibilities, we observed that 0.01 M (1910 mg/l) NTA disodium salt was not readily attacked by sodium hypochlorite in 3 x molar excess at moderate temperatures. NTA persisted in such reaction mixtures for long periods (days) without appreciable conversion to other products.

Conversely, IDA disodium salt (0.05 M, 6650 mg/l) reacted almost instantaneously with 3 x excess (11,200 mg/l) hypochlorite. These concentrations are, of course, at least three orders of magnitude higher than would be expected in sewage treatment plants. Thin-layer chromatography showed rapid formation of a less polar product. The material had oxidizing properties (shown by starch-iodide spray), as would be expected for an N-chloro derivative. The evidence is consistent with a reaction:

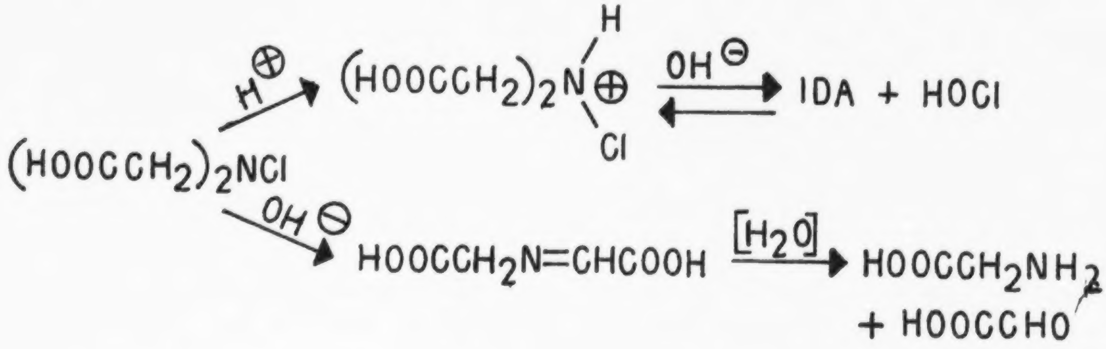
$$(\text{Na OOCCH}_2)_2\ \text{N - H} \xrightarrow{\text{Na OCl}} (\text{Na OOCCH}_2)_2\ \text{N-Cl} + \text{NaOH}.$$

Efforts were made to separate the reaction mixture by preparative TLC, column chromatography, and crystallization, but extensive decomposition occurred. In one preparative TLC experiment, a small amount of pleasant-smelling oil, giving a positive 2,4-dinitrophenyl-hydrazine test (indicative of a carbonyl group) and also having a strong carbonyl band in the infrared spectrum, was isolated.

Attempts to prepare an ester of the N-chloro compound were not successful. Methods requiring acid catalysis gave IDA esters; basic and neutral methods led to extensive decomposition or considerable recovery of starting material.

Attempts were made to prepare the N-chloro compound at pH 2,7, and 12. At pH 2 or 7, the compound could be made in about 50% yield as shown by immediate treatment of the reaction mixture with KI. At pH 12, only 5% conversion was demonstrated. However, after standing for 17 hr., the compound was 93-99% destroyed in all samples.

The instability of the compound and the formation of IDA or a carbonyl compound on its decomposition can be rationalized by the following reaction mechanisms:



The results of the experiments suggest that N-chloro IDA, even if formed at measurable rates at the far lower concentrations of IDA and available chlorine to be expected in sewage plants, would probably not persist for long periods in receiving streams since it has both acidic and basic pathways available for its decomposition. Other routes not investigated (biological degradation or reactions with ferrous ion, polyphenols, or other readily oxidizable substances) might also contribute to its destruction in natural waters.

Photooxidation of NTA and IDA

Preliminary studies showed that NTA and IDA underwent rose bengal-sensitized photooxidation. Singlet oxygen, the reactive intermediate in this reaction, has been shown to be formed upon irradiation of various naturally occurring pigments such as chlorophyll, riboflavin, and "dissolved organic matter." (85) Products identified from the NTA photoreaction were IDA, formaldehyde, and glycine; the photooxidation of IDA also produced formaldehyde and glycine. Some metal-NTA complexes have previously been shown to undergo photooxidation (without the presence of sensitizer), with production of IDA, formaldehyde and CO_2 (37,66). The rates of sensitized photodestruction of NTA and IDA were quite slow, with half-lives of 10-15 days. Accordingly, photooxidation probably does not play a major role in NTA destruction in aqueous solution.

ALGAL STUDIES

Two series of experiments were carried out in order to determine the effect of NTA on algal communities. The first series of experiments was to determine the effect of NTA on the structure of algal communities and the kinds of species composing them; chlorophyll a/c ratios, which reflect the relative abundance of diatoms to other algae; ^{14}C uptake (measured as DPM per microgram of chlorophyll a) to determine photosynthetic activity; and the effect of NTA on the accumulation of metals in the biomass. These experiments were carried out in the winter and then repeated during the summer months in order to determine the effects of natural temperature and light variations.

The second series of experiments was to determine the effects of NTA on the uptake of heavy metals by algae. The same measurements used above to determine change were again made.

Effects of NTA on Algal Communities

The effects of 2.0 and 20.0 mg/l of NTA were studied. In the first experiment in November-February, the amounts of NTA during the course of the experiment varied considerably (Table 5). In the June-September experiments, the concentrations were more nearly constant (Table 6). Average daily minimum and maximum temperature were: November-February, 5.5-8.4°C; June-September, 18.7-22.2°C; March-April, 6.8-12.1°C. Values for pH fluctuated between 7.4 and 8.5 in the November-February and June-September experiments, and 7.3 and 7.9 in March-April.

Chemistry of water. The background chemistry of the water in these series of tests (Tables 7 and 8) was very similar. In the November-February and June-September tests, the $N\text{-}NO_2$ and $N\text{-}NH_3$ increased in the NTA experiments, particularly at the concentration of approximately 20 mg/l. This was probably due to the degradation of the NTA to NH_3 and subsequent oxidation.

Sodium also increased when approximately 20 mg/l NTA was used, because it was introduced as a di- or tri-sodium salt. Zinc seemed to be a little higher in the June-September tests.

Metals in algal biomass. Concentrations of calcium, magnesium, sodium and potassium in the algae showed no consistent trends between summer and winter experiments whether exposed to NTA or not (Tables 9 and 10). However, zinc concentrations tended to increase in the control and decrease in the 2 and 20 mg/l tests in these experiments. There was no dramatic increase in copper concentration in NTA exposed algae; whereas this was observed in the controls in the winter and summer experiments. In these experiments, iron tended to decrease in all treatments and controls. Iron accumulation may have been affected by the presence of NTA, however, for the decrease in the control was not as great. The manganese data, although variable, suggests that accumulation occurred both in the controls and NTA exposed communities.

Thus, we see that the trend of decrease in many of the metals was about the same in 2.0 and 20.0 mg/l during the course of the experiments. Likewise, we see the trend of increases was about the same in the control and the various NTA experiments but increases are often not as large in the NTA tests as in the controls.

The irregular accumulation in the biomass of various chemicals, even in the controls, is probably largely due to the different growths of various algae in the experiments.

Characteristics of the Algal Communities (November 14, 1974 to February 13, 1975)

Microscopic examination of the algal communities on December 10th and December 26th showed a diatom dominance in all experiments (Table 11). *Stigeoclonium protonema* was common in the controls on both dates; the blue-green alga *Schizothrix calcicola* became common, and *Microcoleus vaginatus* was infrequent to frequent. The conditions in the experiments in which the average NTA concentration was 1.61 mg/l showed diatom dominance similar to the control. The blue-green alga *S. calcicola* became common as in the controls. In one of the experiments (Box 1) *Scenedesmus* sp. became frequent to common. At approximately 20 mg/l of NTA, although diatoms remained dominant, the diversity was reduced and *Achnanthes lanceolata* seemed to be in poor cytological condition on December 10th. The blue-green algae, as in the control, at the end of the experiments were common. This similarity in general structure of the algal communities is evidenced by the chlorophyll a/c ratios (Table 12). The DPM of ^{14}C per microgram of chlorophyll *a* seemed to be somewhat less at 20 mg/l NTA, but because the activity of the replicates was variable it is difficult to draw any conclusions.

Analyses of the structure of the diatom communities as indicated by the truncated normal curve model indicates, as did the direct observations during the course of the experiment, that the structure of the diatom community was about the same in the control, approximately 2 mg/l, and approximately 20 mg/l NTA experiments. As seen in Table 13, the number of intervals of the curve covered, sigma squared, the number of observed species, and the number of species in the mode seemed to be quite similar and were not significantly different. There did tend to be a decrease in the number of observed species and the number of species in the mode, and an increase in sigma squared at the end of all experiments when compared with the start of the experiments at approximately 20 mg/l NTA. In previous experiments this trend has been observed when the invasion rate is reduced as happened in these experiments by recycling of water (44). When one looks at the more common species (Table 14) it is evident that *Achnanthes lanceolata* became a larger percent of the community in approximately 20 mg/l NTA when compared with the percent dominance at the beginning of the experiment than in the control and approximately 2 mg/l NTA. It is also evident that *Nitzschia kutzingiana* tended to increase at approximately 20 mg/l of NTA as compared to the control. The growth of *Melosira varians* seemed to increase more in the NTA tests than in the control. The growth of Nitzschia fonticola seemed to be less in approximately 20 mg/l experiment than in the approximately 2 mg/l and the control.

It should be noted that these are only very gross generalizations, because the sizes of the populations of the species at the start of the experiment have a significant effect as to whether that species becomes dominant. It is only the long length of these experiments that allows one to tentatively draw these conclusions, as over time the effect of the treatment on reproduction tends to outweigh the size of the original population.

Characteristics of the Algal Communities (June 20, 1975 to September 3, 1975)

As seen in Table 15, the algal communities in all of the test boxes were dominated by diatoms at the end of seeding (July 2nd). The green alga Stigeoclonium lubricum was present in all of the boxes and was frequent in Boxes 4 and 6. The blue-green alga Schizothrix calcicola was rare to frequent in all of the boxes at the beginning of the test experiment. Achnanthes lanceolata and Melosira varians were common to very common. Cocconeis placentula was common in the controls and Boxes 4 and 6, but was not noted in Boxes 5 and 7. On July 31st some shifts in the communities had occurred. In Boxes 5, 7, 4, and 6, Cocconeis placentula was common but in poor condition. In Boxes 5 and 7 (1.94 mg/l) it was pale in color whereas in Boxes 4 and 6 (19.2 mg/l) it was a deep golden brown, which condition had been observed previously at high NTA concentrations. Melosira varians had increased in all tests and the control. Achnanthes minutissima had become common to very common in Boxes 5, 7, 4, and 6, but not in the control. At the start of the experiments it was not common in any of the boxes. Spirogyra sp. was very common in the control, in poor condition but frequent in Boxes 5 and 7, and not present in Boxes 4 and 6. The blue-green alga Schizothrix calcicola was common in Boxes 4 and 6, rare in Boxes 5 and 7, and frequent in the control. These variations in abundance, particularly in the control and Boxes 5 and 7, are probably not significant. Microcoleus vaginatus was never common.

Detailed examinations of the diatom communities at the end of seeding show that all of the communities were fairly similar as seen in Table 16. Analysis by the end of the experiment, two months later, indicates a normal sequence of events in which diversity does drop in all boxes when invasion of new species is cut off by recycling of water, with no significant differences occurring between boxes. In all boxes σ^2 or the variance in population sizes increased as the numbers of species dropped.

The analysis of the diatom communities from the detailed truncated normal curve studies (Table 17) at the end of seeding substantiated the observations during the course of the experiment--that is, *Achnanthes lanceolata* was the most common species. However, the common occurrence of *Cocconeis placentula* in the control and Boxes 4 and 6 was not as evident. It may have been that these diatoms were not well scraped from the slides, as they stick very tightly to the surfaces of the slides. Since *Navicula secreta* var. *apiculata* is very hard to identify when it is alive, it is not surprising that it was not recorded in the cursory examinations. It is very probable that *Gomphonema parvulum* and *Cymbella cuspidatum*, which were observed to be frequent to common on some of the slides, may have been lost in the transfer to boxes and final cleaning. These diatoms were growing on jelly stalks and were fairly loosely attached to the slides. However, the general condition of the diatom flora in the detailed readings is quite similar to that from the observations. This analysis at the end of the experiment shows normal shifts in the distribution of the more common diatom species. Particularly evident is the reduction in dominance of *Achnanthes lanceolata* and the increase in dominance of *A. minutissima*. Differences between boxes at this time are again not significant.

As seen from Table 18, the percent volatile weight was quite similar in all of the analyses or in all the boxes at the end of seeding and at the end of the experiment. At 30 days after the seeding the volatile weight seemed to be a little less in the test boxes. At the end of the experiments the ash-free dry weight averaged less in the test boxes. A reduction of the chlorophyll a/c ratio is evident at the end of the experiment as compared with the control, which reflects a lesser abundance of algae other than diatoms. The primary productivity analyses done at the end of the experiment show high variance between the duplicate boxes, but the averages would be quite similar. The phycocyanin evidence of blue-green algae increased most in one of the control boxes and one of the 2 mg/l NTA boxes. This was not in accord with the microscopic examinations. This difference may be due to some unknown error in the analyses or due to greater amounts of *Microcoleus vaginatus* as compared to *Schizothrix calcicola*. The former were larger filaments and the cells contained more pigment.

In conclusion, one can say that the results showed similar trends in the June-September experiments as in the November-February experiments. In the approximately 20 mg/l experiments algal growth as measured by the ash-free dry weight was somewhat less than in the controls at the end of the June-September experiment. This was not evident in the November-February experiments.

Whereas during the November-February experiment the chlorophyll a/c ratios were quite similar in all boxes at the end of the experiment, there was a decided decrease in the approximately

20 mg/l experiment in June-September and a smaller but not as low a ratio of chlorophyll a/c in the approximately 2 mg/l boxes. These differences can be attributed to two causes (1) the NTA concentrations were much more constant in the June-September experiment than in the November-February experiments and therefore the algae were consistently exposed to more NTA; and (2) seasonal effects.

The only time a dramatic shift in species was observed was in an initial screening experiment conducted to observe the effects of NTA above the level of 20 mg/l. An achieved concentration of 221.5 mg/l maintained over 30 days led to the replacement of the inital diatom-dominated community by blue-green algae (*Schizothrix* and *Microcoleus*). This condition was verified by pigment analyses. Biomass of the 200 mg/l slides were greatly reduced as compared to controls and communities exposed to approximately 2.0 and 20.0 mg/l NTA.

Experiments with NTA as a Chelator of Heavy Metals

These experiments were carried out in March and April, 1975. As with the previous group of experiments, the water was medium soft, rich in nitrogen and phosphorus in concentrations typical of eutrophic conditions (Table 19). Silica was in abundant supply. The other trace metals and cations and anions were in the proportions expected in a soft water rural stream receiving non-point discharges of nutrients. As seen in Table 20, an average of about 1.57 mg/l NTA was maintained in the NTA-Cu tests, and about 1.37 mg/l NTA was maintained in the NTA-only tests. A low level of NTA contamination was found in the control tests (0.02 mg/l) and in the Cu only test (0.01 mg/l). The identity of the trace contaminants as NTA was confirmed by GC-MS analysis. The source of the NTA was probably filters containing paper made from Canadian pulp.

Microscopic observations of the algal communities showed that in the control, NTA, and NTA plus copper throughout the experiment the flora was dominated by diatoms (Table 21). *Navicula viridula* var. *avenacea* was the most common species. *Achnanthes lanceolata* and *Nitzschia palea* were common to very common in the NTA test and frequent to common in NTA and copper test. In the control, *Microcoleus vaginatus* became rare and *Stigeoclonium lubricum* became fairly common at the end of the experiment. In the NTA tests at the end of the experiment, *M. vaginatus* was rare and *S. lubricum* was common. In the copper alone, we found that the diatoms were dead by March 22nd, roughly one week after the start of the experiment, and gradually *M. vaginatus*, *Schizothrix calcicola*, and *Stigeoclonium lubricum* constituted the algal flora.

It should be noted that the concentration of copper was that which previous experimentation indicated would be toxic or almost completely toxic to diatoms. As can be seen by these results, the NTA chelated the copper and prevented the shift to blue-green algal dominance after the death of the diatoms. These results are also supported by a more detailed study of the diatom community in which the truncated normal curve was generated. We see from Table 22 that in all tests except those that had only copper (in which all diatoms died), the height of the mode was quite similar as were the number of observed species. Sigma squared tended to be greater in the NTA and NTA plus copper than in the control. The number of observed species averaged about the same, and the intervals covered by the curves were similar (Table 23). The more common species equal or greater than 10% in the communities were very similar. Thus there is no evidence that there was a severe alteration in the structure of the diatom community so long as copper was chelated by NTA.

When one compared the diatom communities in the NTA tests in March and April with previous experiments with approximately 2 ppm NTA, it is evident that the number of species at the end of seeding was less than in the two previous experiments; also the height of the mode was less. At the end of the experiment the characteristics of the curve were quite similar to previous experiments.

The observations during the experiment and the detailed readings coincide for *A. lanceolata* and *N. viridula* var. *avenacea*. For species that were frequent in occurrence, one would not expect them to be $\geq$ 1.7% of specimens identified. The similarities of the communities in the control, NTA, and copper plus NTA was also manifested by the chlorophyll a/c ratios (Table 24). Since the diatoms were dead in the copper experiments, no ratio could be determined. The percent volatiles were similar on the control, NTA, and copper plus NTA experiments. It was much less as one would expect in the copper experiments, where the diatoms were dead. The ^{14}C uptake was quite similar in the control, NTA, and copper and NTA experiments being greatly reduced in those experiments where copper was alone. Thus we see that all of the parameters used point to the similarity of the communities in the control, NTA experiments, and copper plus NTA. They also point to the great difference in those experiments where copper only was present at approximately 100 μg/l.

Phycocyanin concentration was quite similar in all of the boxes (Table 24). This probably means that in those tests where diatoms were dominant the blue-green algae were harder to see and therefore were not so actively recorded; whereas in the copper experiments where everything was dead except blue-green algae they were very evident and thus the observations would indicate an increase in these boxes. Another explanation may be due to the fact that *Schizothrix calcicola*, which became very abundant, is a very small filament and one could have an increase many fold in filaments without having much increase in phycocyanin.

Thus, the phycocyanin is a very rough measurement of the number of filaments present. However, it does indicate that blue-green algae were in all the boxes as did the direct microscopic observation.

The ash-free dry weight, as one would expect, is much less in the tests with copper alone. This weight was little less in the NTA and NTA plus copper tests than in the control.

The experiments with NTA and copper showed increased growth and diatoms in good condition in the control, NTA, and NTA plus copper. In copper alone, the diatoms were in poor condition after one week's exposure and were dead at the end of the experiment. However, *Schizothrix calcicola* was abundant at the end of the experiment as was a yeast, and in the reduced biomass these were the dominant forms.

Copper in the biomass (Table 25) in the control was first up a little, then down; in NTA alone it showed no significant change; whereas, in the NTA plus copper it increased 2.4 times at the end of the first week and approximately five times by the end of seeding. As one would expect in copper alone, it increased 13 times at the end of one week after seeding and 42 times by the end of the experiment.

In conclusion, NTA greatly influenced the uptake of copper by the algal populations and prevented the toxic effect of copper to the algal community. It prevented the decrease of ^{14}C uptake and maintained the chlorophyll a/c ratios similar to those in the control. There seemed to be also an effect of the presence of NTA on the absorption of other metals (one week after the experiment started). In some cases the absorption was decreased and in others it would appear that it was enhanced (Table 25).

BACTERIAL STUDIES

Bacteria are the most important organisms in the ecosystem involved in the degradation of dissolved organic nutrients with the resultant generation of mineralized end products (for example, carbon dioxide from organic carbon compounds). Experiments to determine the rate of degradation of NTA by the natural stream flora at different seasons of the year were therefore done. Degradation was assayed under aerobic conditions in these experiments. Another focus of experiments conducted under this program was to see whether the degradation of other substrates would be affected by the presence of NTA-metal chelates.

NTA Degradation Studies

Experiments with pure cultures of NTA degrading bacteria. Preliminary experiments to evaluate procedures were conducted with NTA degrading isolates; one (a *Pseudomonas* sp.) obtained courtesy of Dr. James Tiedje (Michigan State University), the other was isolated from White Clay Creek. Organisms cultured in NTA broth were harvested while in the log phase of growth, washed twice in phosphate buffer (0.01 M, pH 7), and transferred to each of six biometer flasks to provide a final concentration of ca. 10^{10} cells/ml in 15 ml. NTA mineralization was measured for 3 hr. and cells were recovered at the end of the experiment by filtration. The results with one isolate (T-1) are shown in Fig. 10. Active mineralization was evident although only 10 percent of the added radioactivity (170,000 DPM) was recovered as ^{14}C-carbon dioxide with this isolate after 3 hr. The White Clay Creek isolate "216" mineralized 20 percent of the added radioactivity in 90 min. (ca. 10^9 organisms/flask). Negligible activity was associated with the cells.

Experiments with natural bacterial communities. One experiment was conducted between March 12 and April 24, 1975. During this time, the stream temperature ranged from 2.0 to 15.0°C (mean ± standard deviation 8.6 ± 1.8°C). Assays were conducted at 9°C. NTA concentrations in the microcosms are shown in Table 26. NTA was detected in seven of 41 control samples: in six the concentrations were in the range of 0.003-0.016 mg/l, but the seventh contained 0.092 mg/l. This periodic contamination was traced to filters which were used to reduce the silt load and algal cells in incoming water during the recycle portion of the experiment. NTA was subsequently identified by gas chromatography-mass spectrometry in the leachate from the pressed paper filters.

Data from a typical series of assays from this experiment is shown in Table 27. In most instances there is good replication among the five experimental flasks. There is a striking difference in degradation rate between the experimental and dead cell control flasks for populations exposed to NTA in microcosms. For populations from the control (unexposed) microcosms, activity is at background level.

Biodegradation rates before and after exposure to NTA are shown in Fig. 11. The intermittent exposure by contamination of populations in the control microcosms to NTA never led to NTA degradation at an elevated rate such as that occurring in the other microcosms. The development of degradation capability was concentration dependent, with more rapid development being correlated with higher concentrations. At the end of six weeks there was a 250-fold difference in degradation rate 0.4 - 100 ng/hr/cm^2) over the 1000-fold range at which degradation was tested.

Between six and eight weeks the temperature in the microcosms rose (mean ± standard deviation, for this period 12.3 ± 0.95°C). At the eighth week, the degradation rate leveled off or declined between 0.2 - 20 mg/l. Slight increases were observed for the 0.02 mg/l and control populations.

This may represent the achievement of a maximal degradation rate by the flora. Alternatively, the rising water temperature may have affected the flora detrimentally. If a flora with higher temperature optimum was developing, and if the assay temperature (9°C) was not limiting, the results support the contention that during a time of rapid ambient temperature change, when organisms with a new temperature optimum are developing, the ability of the system to assimilate a given input would be diminished until the new flora become established (85).

The number of cells capable of NTA degradation increased on the coverslips through the fourth week of the experiment and declined into the sixth week, as is shown in Fig. 12. Increases were observed at some concentrations between the sixth and eighth weeks. Greatest increases were obtained at the highest NTA concentrations.

The experiment was repeated in July and August, 1975, when stream temperatures were in the range of 14.5-22.4°C (mean $\pm$ standard deviation, 18.4 $\pm$ 0.9°C). Assays were performed at 18°C. NTA concentrations in the microcosms are shown in Table 28. The development of NTA degrading ability was again seen, but this development was faster and reached a higher level of activity than in the earlier experiment (Fig. 13). This is presumably the result of warmer temperature since NTA concentrations were similar. The activity of the populations exposed to 0.2 - 20 mg/l (actually 0.17 - 20.1 mg/l) appeared to plateau by the fifth week of exposure. At the higher concentrations (2.0 and 20 mg/l), there was approximately a tenfold increase in decomposition rate in the summer experiment when compared to the early spring experiment, but this was not the case for the populations exposed to approximately 0.02 or 0.2 mg/l. The populations in the control microcosm were intermittently exposed to NTA, and the exposure appeared sufficient to bring about an increase in degradation rate by the second and third weeks of the experiment (Fig. 13). Numbers of NTA degrading organisms increased more than a hundredfold on exposure to 2 and 20 mg/l (Fig. 14). Lesser increases were observed at the lower concentrations.

The complete degradation of NTA yields carbon dioxide, ammonia, and water. In a supplemental experiment conducted in November, 1974, NTA was metered into four microcosm systems to establish concentrations of approximately 100 mg/l and the water was assayed over time for concentration of ammonia-N, nitrite-N, and nitrate-N. The temperature during the experiment ranged from 6.9 to 11.2°C.

Ammonia-N concentrations increased owing to the decomposition of the NTA and subsequently, the nitrite-N concentrations increased (Fig. 15A). Nitrate-N concentrations did not show much change. The ambient levels of 2-3 mg/l probably overwhelmed the maximum additional amount of 0.1-0.25 mg/l that would have resulted from the oxidation of all the nitrite-N. Similar results have been noted in other experiments. Measurements of ammonia-N and nitrite-N in the 20 mg/l NTA system of the March-April, 1975, decomposition experiment discussed above, showed an increase and subsequent decline in concentration of ammonia-N and nitrite-N (Fig. 15B). In another experiment, (August, 1974, 16-22°C) increases in nitrate-N were also seen but NTA concentration in this experiment appoximated 200 mg/l (Fig. 15C).

In another study of NTA degradation, ^{14}C labeled NTA was added to a recycle microcosm system in which NTA had been maintained at a concentration of approximately 2.0 mg/l for approximately 35 days. During the three-day experimental period, the average concentration was 2.2 mg/l (n = 5, range 1.3-3.5 mg/l). Temperature ranged from 8.0-10.2°C. The results are shown in Fig. 16. Degradation of the ^{14}C-NTA began immediately with the subsequent release of $^{14}CO_2$ to the water. After 12 hours, approximately 24 percent of the radioactivity introduced into the system was lost as $^{14}CO_2$. The slope of the empirical line differs from the slope of the line showing the theoretical loss from dilution alone. A semi-logarithmic plot of the data was made, and regression lines were fit. The difference in the slopes of these lines was used to calculate a half life of NTA in the system resulting from bacterial activity ($T_{1/2}$ = 14.4 hr.). The degradation estimate from slopes of lines is probably conservative because any non-volatile products of decomposition would be considered as NTA.

In summary, these experiments with natural stream bacterial populations showed (from direct measures of NTA mineralization)that an NTA degrading flora will develop at different seasons of the year and degradation will occur under aerobic conditions. Degradation capability was correlated directly with concentration of exposure, duration of exposure, and with temperature. Increasing temperature and concentration shortened the lag time before degradation occurred at an elevated rate. Our findings are in keeping with those of others (61,62,70,82) in which NTA degradation (measured by disappearance) in water was also demonstrated.

Preliminary experiments in which NTA degradation was followed under microaerophilic (0.27 ± 0.31 mg dissolved oxygen/l) and oxygen free (<0.01 mg dissolved oxygen/l) were also performed. Samples were sparged twice daily with helium to lower oxygen concentrations in the first instance. To provide oxygen-free conditions samples were incubated under H_2-CO_2 in anaerobic jars and measurements and manipulations were made in a glove bag under an argon atmosphere. Sediment microbial populations degraded NTA from initial concentrations of 8 and 140 mg/l in the presence of low oxygen but not from 200 mg/l in its absence. In one experiment lasting 3.5 months the redox potential (E_{cal}) in the oxygen free environment was -380 to -480 mv, conditions under which NO_3^- would not be present but $SO_4^=$ would be present to some degree. Enfors and Molin (19,20) noted NTA degradation in an anaerobic environment when NO_3^- was present at 1000 mg/l to serve as a terminal electron acceptor. Our results, although preliminary, indicate that the redox potential will be an important determinant of NTA degradation in anaerobic environments and further studies should be done.

NTA-Metal Chelates and Bacterial Activity

Studies were done to examine the effectiveness of NTA in protecting bacteria from the toxicity of selected toxic metal ions. Heterotrophic bacterial populations from the White Clay Creek and NTA degrading isolates were used in these studies. The metabolism of ^{14}C labeled glucose was used to measure effect. Data from a typical experiment with stream populations is presented in Fig. 17. The metal ions tested (zinc, cadmium, copper) were toxic although to varying degrees. Protection was afforded by the NTA-metal chelates.

The results with mixed heterotrophic populations cultured from stream communities showed that the toxicity of cadmium, zinc, lead and nickel varied considerably from experiment to experiment with inhibition ranging from 18-84% (Table 29). Inhibition of glucose incorporation by exposure to metallic ions was similar whether expressed as a percent of the activity of controls (metal/control) NTA exposed cells (metal/NTA) or cells exposed to NTA-chelated metal (metal/chelate). Because the composition of the inoculum undoubtedly differed between dates a strong inverse relationship between inhibition and inoculum level was not apparent. Copper toxicity, however, was consistently pronounced (Table 30) and inhibition of glucose metabolism ranged from 75-90%. Exposure to metals as NTA-chelates conferred protection from toxicity. (Compare metal/control with chelate/control columns in Tables 29 and 30). In two experiments (lead, 10/28; nickel 9/16) the metal concentrations were increased from 1 mg/l to 20 and 30 mg/l respectively, but even at these levels, protection was obtained when the metal was chelated. Glucose incorporation by mixed stream organisms in the presence of the chelate was generally similar to that of the control; in 75% of the experiments, activity was ± 25% that of the controls (chelate/control in Tables 29 and 30). Some additional experiments performed with cells that had been starved prior to glucose exposure gave results essentially the same as those for unstarved cells shown here. In experiments with lead chelates the acetate salt was used. A separate experiment showed no effect on glucose incorporation from acetate added in the amount that would be freed on NTA-chelate formation.

Simulation models (14; Chance, unpublished) suggest that 4.0 ppm NTA added to White Clay Creek water without chelation to a specific metal yielded the iron or calcium chelate. Glucose incorporation in the presence of these NTA salts was occasionally stimulated or repressed, but was ± 25% of incorporation in controls in 7 of 18 experiments (Table 31).

Other experiments also showed NTA neither dramatically stimulated nor depressed the utilization of glucose by mixed populations. In these assays, ^{14}C labeled substrate was added initially and NTA was added to provide final concentrations of either 2 or 4 mg/l either initially or after a 48-minute preincubation during which time a baseline metabolic rate was determined for the community. With this approach, a change in rate of ^{14}C glucose metabolism before and after NTA addition would be indicative of an effect. Both incorporation and mineralization rates were monitored, and the results (Fig. 18) show no striking rate change on NTA addition compared to controls. Again, NTA was probably present as the iron or calcium chelates, the predominant form in White Clay Creek water. Other experiments conducted similarly but with natural populations colonizing microscope cover glasses suggested slight stimulation of ^{14}C glycine mineralization by 20 mg/l NTA and of ^{14}C glutamic acid mineralization by 2% NTA; but these NTA concentrations are unexpected in nature.

NTA degrading isolates were used in short-term experiments. Copper consistently reduced incorporation of ^{14}C glucose by approximately 90% (Table 32). Inhibition of glucose incorporation by cadmium and zinc ions varied from 41-85% and 8-47% respectively. The NTA-chelate of zinc protected organisms from toxicity (compare metal/control with chelate/control). However, in one experiment with cadmium and in those with copper, incorporation in the presence of the chelate was also considerably reduced compared to the control. Perhaps metabolism of the chelate by the NTA degrading isolate released metal ions in concentration sufficient to be toxic to these populations unacclimatized to copper exposure. It is also of interest that glucose incorporation by isolate "216" was reduced when NTA was present as the Ca- or Fe-chelate, suggesting the presence of NTA lowered the utilization of an alternative energy and nutrient resource.

At the end of the experiment in which stream communities were exposed to 30 μg Cu/l and 2 mg NTA/l or 2 mg NTA/l with no copper (pp.49-64), bacterial communities on silt from the ecosystem stream bottom were tested for their ability to degrade ^{14}C NTA. Populations from the Cu-NTA stream were tested for ability to degrade the Cu-chelate and those exposed to NTA for their ability to degrade the Fe- or Ca-chelate. Although there were no statistically significant differences between streams when NTA degradation activity was normalized for bacterial numbers, activity normalized for sediment weight was lower for the Cu-chelate than the Ca- and Fe-chelates. The results of two trials are shown here:

	µg NTA degraded/µg sediment (n, $\bar{x}$, s.d.)	
Exposure	Trial 1	Trial 2
Control	5, 145, 137	5, 47, 49
NTA/Cu	5, 110, 49	5, 147, 37
NTA	5, 316, 166	5, 864, 575

Differences in trial 1 were not significant statistically, but in trial 2 the activity of the NTA exposed population was significantly greater than the control or the population that had been exposed to NTA and copper for the previous four months.

Exposure over longer time periods was studied in experiments in which the respiration of cell suspensions was used to measure effect. Results are presented in Table 33. In all instances exposure to Cu, Cd, Zn, and Hg ions stopped respiration, but respiration usually resumed by 2.5 hr. A 5:1 NTA:metal ratio provided protection from the toxic effects of metal ions in all experiments but the one with mercury. Cell numbers increased during experiments, and after 20 hr., a multiplicity of types were observed (long and short bacilli, cocci, and some yeasts) in many flasks. In all instances, the metal concentrations associated with cells at the end of the experiment were lower when the metal was added as the NTA chelate than when added alone.

In summary, our studies on the effect of metals or NTA-metal chelates on the metabolism of other organic compounds by natural stream bacterial populations demonstrate that NTA afforded protection from Cd, Cu, Zn, Ni, Pb, and Hg ions which were toxic to varying degrees at 1.0 mg/l. Accumulation of metals was not accelerated by NTA in the one experimental series in which this was measured and additional experiments on the sequestering of metals by NTA should be done. Additional work with NTA degrading isolates which may release metal ions in concentrations sufficient to be toxic and inhibit microbial activity is also needed.

EFFECT OF NTA AND COPPER IN ECOSYSTEM STREAMS

This study was performed to assess the impact of NTA on entire communities in experimental streams in which natural conditions could be closely simulated. From our studies in microcosms and the literature, we did not expect NTA to exert acute effects on the biota and most emphasis was placed in this aspect of the program on the chelation of metal ions by NTA. To emphasize this, 2 mg/l NTA was added along with 30 µg/l copper (as copper sulfate) to one stream (designated stream II), 30 µg/l copper was added to another (designated stream III), 2 mg/l NTA to another (stream IV), and one stream was the control, which received no additions (stream I). The concentration of copper and four other metals (iron, manganese, magnesium, and zinc) in plant and animal biomass was monitored in order to examine whether NTA, introduced at the suspected upper limit of environmentally realistic concentrations, could alter these over a period of time. The experiment lasted approximately 3 months, from July 13-October 6, 1976. Average water temperature during the study period was 16.9 ± 2.00 ($\bar{x}$ ± s.d., n = 86) and ranged from 10.5 - 22.5°C.

Concentrations of several dissolved inorganic constituents during the study period are presented in Table 34. There was very little difference between streams in concentrations of the ions measured. Concentrations of NTA and copper are shown in Table 35. The mean concentrations of both NTA and copper actually achieved were extremely close to those desired over the three month experimental period.

Within the first week of the experiment, the community in the stream receiving copper alone was dramatically affected. Anacharis populations had a bleached appearance and eventually disappeared as a result of decomposition and sampling pressure. Algal populations sloughed off leaving the sediments bare for approximately two weeks. At a later time, approximately three weeks into the experiment, the green alga Ulothrix developed macroscopically visible populations. Planarian worms which were prevalant on the Anacharis also disappeared in the copper treated stream. The streams receiving NTA and copper or NTA alone appeared little different from the control stream, suggesting NTA had no acute effects and, in addition protected the biota from copper toxicity.

The concentrations of copper, iron, zinc, manganese and magnesium ions in the tissue of algal communities, Anacharis sp., Lemna sp. and Planaria sp. and Tubifex sp. were monitored through the experiment. The data for each sample type were used in a two-way analysis of variance to test for significant difference between times and streams. The ANOVA's invariably were invalidated by highly significant two-way interaction effects. Therefore, the data were examined for (1) differences between the first (prior to chemical additions) and subsequent (post treatment) collections for each stream and (2) significant differences between streams for individual collections that occurred consistently. The Scheffe multiple range test (P = 0.05) was used where variances were homogeneous and the Kruskal-Wallis multiple comparison test (P = 0.05) where variances were non-homogeneous. Both tests are conservative with respect to revealing significant differences; the Kruskal-Wallis is more so than the Scheffe. Statistically significant differences in data from post-treatment collections in a given stream were also obtained sometimes but these are not discussed in the interest of clarity. Although we infer biological significance where statistical significance is demonstrated, this may not always be the case.

ALGAE

No material was available for collection from the copper treated stream (III) at sampling times 2 and 3.

Copper (Fig. 19)

Data for collection 5 was excluded from analysis because of small sample size.

Differences between first and subsequent collections in:

Stream I. (Control)	Concentrations of copper in algal communities decreased through time. Collections 6, 7, and 8 were significantly lower than 1 (Scheffe test).
Stream II. (NTA/Cu)	Concentrations decreased through time. Concentrations in collections 7 and 8 were significantly lower than in 1. (Scheffe test).
Stream III. (Cu)	Concentrations associated with the copper tolerant flora (collections 4-8) were significantly increased over those in the algae present at the start of the experiment (Scheffe test).
Stream IV. (NTA)	The concentration in collection 4 was significantly greater than in collection 1 but the weight for collection 4 was smaller than for any other. The coefficient of variation for the data was similar to other data sets but the small sample size may have been a source of error. Concentrations at collections 6 and 8 were significantly lower than at the start. (Kruskal-Wallis test).

Differences between streams at each collection:

Copper concentrations in algae in the streams receiving NTA and the control differed to a statistically significant extent only once (Table 36). At collection 4 the algae in stream IV (NTA) had higher copper levels but as noted above, error may be associated with this data. Copper concentrations in copper tolerant algae from stream III (Cu) were greater than in the algae from the other streams in collections 4, 6, and 7 and in the single determination at collection 8 although the statistical tests used did not always reveal significance. Copper concentrations in algae exposed to CU and NTA (stream II) never differed significantly from the control. This indicates that NTA prevented the uptake of the added copper, since concentrations in algae in stream III were much higher.

Iron (Fig. 20)

Differences between first and subsequent collections in:

Stream I. (Control)	Iron concentrations in the algae showed no significant difference between collection 1 and subse-

	quent samples but for collection 2 which was higher (Kruskal-Wallis test).
Stream II. (NTA/Cu)	No significant differences occurred but for collection 2, which was higher than 1 (Kruskal-Wallis test).
Stream III. (Cu)	Iron concentrations in collections 6 and 7 were elevated over collection 1 (Kruskal-Wallis test).
Stream IV. (NTA)	No significant differences between the first and subsequent collections occurred but for collection 2 which was higher (Kruskal-Wallis test).

Iron concentrations at collection 2 were elevated in samples from all streams, but the coefficients of variation for these data (9-45%) were not greater than for other collections and so the data are not excluded.

Differences between streams at each collection:
No significant difference between streams receiving NTA (II and IV) and the control occurred in six of seven post treatment collections (Table 36). The exception was collection 7 when algae in stream II (Cu/NTA) contained more iron than those from the control, but variance for these samples is large. NTA exposure appeared to have no effect on the iron content of algae. Concentrations of iron in algae in the copper treated stream (III) tended to be higher and were significantly elevated over the control in two collections (5 and 7).

Zinc (Fig. 21)

Data for collection 5 was excluded because small sample size may have introduced analytical error. Data for stream I at collection 4 had a coefficient of variation of 166% (compared with the 8-30% associated with most data) and was also eliminated from analysis.

Differences between first and subsequent collections in:

Stream I. (Control)	Zinc concentration in collection 3 algae was significantly greater than in collection 1 material (Kruskal-Wallis test).
Stream II. (NTA/Cu)	Collections 4, 7, 8, were significantly lower than collection 1 (Kruskal-Wallis test).
Stream III. (Cu)	No significant difference in concentrations between the first and subsequent collections (Scheffe test).
Stream IV. (NTA)	At collections 2, 6, 7 and 8, zinc concentrations in the algae were significantly lower

than in collection 1 (Scheffe test).

Differences between streams at each collection:
Prior to treatment (collection 1) differences between streams (I and IV) were observed.

No statistically significant differences in zinc concentration in algae from the control and NTA treated streams (II and IV) occurred at collections 3, 6 and 7 (Table 36). When compared to the control, algae in these streams had significantly lower zinc concentrations in collection 2, and in collection 8 the algae in stream II did. In collection 7, algae in the copper treated stream contained significantly more zinc than algae in the other streams.

Concentrations of zinc in algae in stream IV (NTA treatment) declined through time and were significantly lower at the end of the experiment than at the start. Although statistically significant differences between streams were not observed consistently, the decreasing trend in concentration over time in streams receiving NTA suggests that zinc concentration in algae biomass should be monitored in future studies with NTA.

Manganese (Fig. 22)

Differences between first and subsequent collections in:

Stream I. (Control)	No significant differences in manganese concentrations in algae were found between collection 1 and subsequent collections (Kruskal-Wallis test).
Stream II. (NTA/Cu)	At collections 2 and 6 there was significantly less manganese in algae than at collection 1 (Kruskal-Wallis test).
Stream III. (Cu)	Concentrations in collections 5 and 6 were significantly lower than in collection 1 (Kruskal-Wallis test).
Stream IV. (NTA)	At collection 2, manganese concentration was significantly lower than at collection 1 (Kruskal-Wallis test).

Differences between streams at each collection:
Concentrations in algae in the treated streams were not significantly different from the control for collections 2, 3, 4, 6, and 7 (Table 36). In two collections (5 and 8) manganese concentrations in algae from stream IV (NTA) were significantly elevated over the control, but in collection 8 the concentration in algae from the NTA/Cu stream (II) was significantly lower than collection 8 in the control. Again, note that differences were observed prior to treatment; at collection 1 stream I differed significantly from streams III and IV. The lack of consistent

differences between streams and the similarity of concentration changes in all streams both suggest that NTA exposure did not affect manganese concentrations of the algal communities studied here.

Magnesium (Fig. 23)

Differences between first and subsequent collections in:

Stream I. (Control)	Magnesium concentrations in algae in collections 2 and 5 were significantly higher than in collection 1 (Kruskal-Wallis test).
Stream II. (NTA/Cu)	In collections 2, 5, and 7 concentrations were significantly higher than in collection 1 (Kruskal-Wallis test).
Stream III. (Cu)	In collections 5 and 7 concentrations were significantly higher than in collection 1 (Kruskal-Wallis test).
Stream IV. (NTA)	Concentration of magnesium in algae in collection 5 was significantly higher than in collection 1 (Scheffe test).

Although significant differences in concentration of magnesium in algae were obtained between the initial and post treatment collections, these occurred in all streams and there was no increasing or decreasing trend in concentration through time.

Differences between streams at each collection:
Magnesium concentrations in the algae from the control and NTA containing streams differed significantly over at collection 7 when algae in stream II (NTA/Cu) had higher levels than algae in the control (Table 36). Overall, it is unlikely that NTA exposure affects magnesium concentration in algae. Concentrations in material from the copper treated stream (III) differed significantly from the control in collections 6 and 7 (Table 36) although standard deviations overlapped at collection 6.

ANACHARIS

No material was collected from stream IV at collection 6 or from stream III following collection 4.

Copper (Fig. 24)

Differences between first and subsequent collections in:

Stream I. (Control)	At collection 4, *Anacharis* had significantly more copper than at collection 1 (Kruskal-Wallis test).
Stream II. (NTA/Cu)	Samples in collection 8 contained significantly less copper than material at collection 1. (Kruskal-Wallis test).

Stream III. (Cu) Concentration in collection 2 was significantly greater than in collection 1 material (Scheffe test) and the single determination at collection 4 was elevated.

Stream IV. (NTA) Collections 6, 7, and 8 contained significantly less copper than collection 1. (Kruskal-Wallis test).

Changes in concentrations of copper in *Anacharis* through time were similar in all streams.

Differences between streams at each collection:
Differences between the control (I) and streams receiving NTA (II and IV) existed in some collections (Table 37) but sometimes concentrations in NTA exposed material were significantly greater and sometimes significantly less than in material from the control stream. Overlap of standard deviations occurred in some instances. Copper concentrations in *Anacharis* in stream III (Cu) were elevated over the control in collections 2 and 4 but not 3 although differences were not statistically significant by the tests used. Differences existed prior to treatment. Stream II differed from stream III and IV.

Iron (Fig. 25)

Differences between first and subsequent collections in:

Stream I. (Control) Iron concentrations in *Anacharis* in collections 3 and 5 were significantly lower than in collection 1 (Kruskal-Wallis test).

Stream II. (NTA/Cu) In collections 3, 5, 7, and 8 concentrations were significantly lower than in collection 1. (Kruskal-Wallis test).

Stream III. (Cu) Concentration in collection 2 was significantly higher than in 1 (Scheffe test).

Stream IV. (NTA) In collections 2 through 8, *Anacharis* contained significantly less iron than in collection 1. (Scheffe test).

Differences between streams at each collection:

Differences existed before chemical additions; at collection 1 stream IV was significantly higher than I (Table 37). Concentrations of iron in *Anacharis* in the copper treatment were significantly elevated over the control in collection 2 and 3, and in the single sample available at collection 4. Although significant differences between one of the NTA treated streams and the control were observed only twice, (Stream IV, collection 4; stream II, collection 7, Table 37) iron concentration in material exposed to NTA tended to be lower than in the control. Iron accumulation in *Anacharis* should also be more thoroughly investigated in any future work with NTA and aquatic organisms.

Zinc (Fig. 26)

Differences between first and subsequent collections in:

Stream I. (Control)	In this stream large variance was associated with collection 1 data and only collection 3 differed from it (Kruskal-Wallis test).
Stream II. (NTA/Cu)	Differences between the first and subsequent collections were not significant (Kruskal-Wallis test).
Stream III. (Cu)	Collection 3 was significantly higher than 1 (Scheffe test).
Stream IV. (NTA)	In collections 2, 7, and 8, *Anacharis* contained significantly less zinc than in collection 1; in collections 3, 4, and 8 it did not (Kruskal-Wallis test). Five extremely high values (range: 1687-2977 µg/g were eliminated from collection 6 data because of suspected contamination.

Differences between streams at each collection:

A significant difference in zinc concentration in *Anacharis* from the control stream and either one or both of the streams receiving NTA was observed in all post-treatment collections but 3 and 6 (Table 37). The tendency was for the concentration of zinc to be lower in *Anacharis* exposed to NTA. No significant differences between material in the control and copper treated stream was observed. As with algae, NTA appeared to lower zinc concentrations in plant tissue and for the *Anacharis* data, statistical significance is present.

Manganese (Fig. 27)

Differences between first and subsequent collections in:

Stream I. (Control)	No significant differences in concentration of manganese in *Anacharis* between the first and succeeding collections occurred but for collection 2 which was significantly lower (Kruskal-Wallis test).
Stream II. (NTA/Cu)	Concentration in collection 2 was significantly lower than in collection 1 (Scheffe test).
Stream III. (Cu)	Concentrations in collections 1, 2, and 3 were not significantly different (Scheffe test) but the single sample at collection 4 had a value of 507 µg/g.

Stream IV. (NTA) No significant difference in manganese concentration in *Anacharis* occurred between collection 1 and subsequent collections but for collection 2 which was lower (Scheffe test).

Concentrations at collection 2 were low is material from all streams.

Differences between streams at each collection:

A significant difference between the control and NTA exposed material was observed only once; in collection 4 concentrations in *Anacharis* exposed to NTA were significantly lower (Table 37). Overall, NTA exposure had no striking effect on manganese concentrations in this plant. Differences between the control and copper treatment were not significant except in the single sample taken at collection 4 in which the concentration was unusually high.

Magnesium (Fig. 28)

Differences between first and subsequent collections in:

Stream I. (Control)	Magnesium concentrations in *Anacharis* were significantly higher at collections 3, 4, and 5 than at collection 1 (Kruskal-Wallis test).
Stream II. (NTA/Cu)	In collection 3, the concentration was significantly higher than collection 1; otherwise, there were no significant differences (Kruskal-Wallis test).
Stream III. (Cu)	The concentration in collection 3 was significantly higher than collection 1 (Scheffe test).
Stream IV. (NTA)	No significant differences between collection 1 and subsequent collections occurred (Kruskal-Wallis test).

Differences between streams at each collection:

A significant difference in magnesium concentration in *Anacharis* from either NTA stream and the control was obtained only in collection 8 (Table 37). Concentration in collection 3 material from the copper treatment was elevated over the control. Under the experimental conditions, NTA exposure did not appear to affect the concentration of magnesium in *Anacharis*.

LEMNA

No material was available from stream IV at collection 8.

Copper (Fig. 29)

Differences between first and subsequent collections in:

Stream I. (Control)	Copper concentration in *Lemna* at collection 8 was lower than in collection 1 (Kruskal-Wallis test).

Stream II. (NTA/Cu) Concentration in collection 4 was significantly greater than in collection 1 (Kruskal-Wallis test).

Stream III. (Cu) Concentrations in pre- and post-treatment material were not different (Kruskal-Wallis test).

Stream IV. (NTA) Concentrations in material from collections 4 and 5 were significantly greater than at collection 1 (Kruskal-Wallis test).

Changes in concentration through time were similar in all streams.

Differences between streams at each collection:
Copper concentrations in *Lemna* from the control and the NTA treated streams were significantly different only in collection 5 (Table 38). Samples from stream I (Control) and IV (NTA) never differed significantly, but in collection 5 material from stream II (NTA/Cu) differed from the control. The *Lemna* taken from the copper (III) stream contained significantly more copper than samples from the control at collection 2 and 4. It is unlikely the NTA treatments used here affected the concentration of copper in *Lemna*. Prior to treatment, streams I and IV differed.

Iron (Fig. 30)

Differences between first and subsequent collections in:

Stream I. (Control) Iron concentrations in *Lemna* in collections 7 and 8 were significantly lower than in collection 1 (Kruskal-Wallis test).

Stream II. (NTA/Cu) Iron concentration in collection 7 was significantly lower than in collection 1 (Kruskal-Wallis test).

Stream III. (Cu) Differences in concentration between collection 1 and subsequent collections were not significant (Kruskal-Wallis test).

Stream IV. (NTA) Collection 4, 5, and 7 contained significantly lower iron concentrations than collection 1 (Scheffe test).

Concentrations in collection 6 were unusually high in samples from all streams.

Differences between streams at each collection:
No significant differences in concentration between samples from control or either NTA stream existed in four post-treatment collections (2, 3, 5, and 7, Table 38). Concentrations in stream IV (NTA) material were lower in two collections (4 and 6) and higher in stream II (NTA/Cu) material at collection 8 although

standard deviations overlap in the latter instance. It is unlikely NTA exposure affected iron concentrations in *Lemna*.

Zinc (Fig. 31)

Data from collection 3 was excluded from analysis because of contamination.

Differences between first and subsequent collections in:

Stream I. (Control)	No significant differences in zinc concentration in *Lemna* occurred between collection 1 and subsequent collections (Kruskal-Wallis test).
Stream II. (NTA/Cu)	Concentrations in collections 7 and 8 were significantly lower than in collection 1 (Kruskal-Wallis test).
Stream III. (Cu)	Concentration in collection 8 was significantly lower than in collection 1 (Kruskal-Wallis test).
Stream IV. (NTA)	No significant differences in zinc concentration in *Lemna* occurred between collection 1 and subsequent collections (Scheffe test).

Differences between streams at each collection:

Significant differences in concentration of zinc in *Lemna* between the control and one or other of the streams receiving NTA were observed in all collections and at collections 4 and 7, the material from both NTA streams differed significantly from the control (Table 38). The trend was for NTA exposure to lower the measured concentration of zinc in *Lemna* biomass. This is the trend noted with algae and *Anacharis*. Copper exposure (III) affected zinc concentration significantly only in collection 2.

Manganese (Fig. 32)

Differences between first and subsequent collections in:

Stream I. (Control)	Manganese concentrations in *Lemna* at collections 6 and 7 were significantly lower than at collection 1 (Kruskal-Wallis test).
Stream II. (NTA/Cu)	Manganese concentration in collection 2 was significantly higher than in collection 1 and in collection 6 it was significantly lower (Scheffe test).
Stream III. (Cu)	Concentrations in all collections but 4 (i.e. 2, 3, 5, 6, 7, and 8) were significantly lower than in collection 1 (Scheffe test).
Stream IV. (NTA)	Concentrations in collections 5, 6, and 7 were all significantly lower than in collection 1 (Kruskal-Wallis test).

Differences between streams at each collection:
Concentrations of manganese in Lemna from streams I and III were significantly different at collection 1. Variable groupings of data were obtained at each collection time and consistent differences between streams were not evident (Table 38). One or both NTA streams differed from the control usually, but the concentrations in the NTA exposed material were sometimes lower and sometimes higher. Copper exposure significantly lowered manganese concentration in Lemna in five of seven post treatment collections.

Magnesium (Fig. 33)

Differences between first and subsequent collections in:

Stream I. (Control)	Concentrations in collections 3 and 5 were significantly higher than in collection 1 (Scheffe test).
Stream II. (NTA/Cu)	Concentrations in collections 2, 3, 5, and 6 were significantly higher than in collection 1 (Scheffe test).
Stream III. (Cu)	In collections 2 and 3 concentrations were significantly higher than in collection 1 (Kruskal-Wallis test).
Stream IV. (NTA)	In collections 2, 3, and 5, concentrations were significantly higher than in collection 1 (Kruskal-Wallis test).

All streams tended to have higher concentrations at collections 2, 3, 5, and 6 compared to the initial values; some differences were significant, others were not.

Differences between streams at each collection:
Magnesium concentration at collection 1 was significantly lower in Lemna from stream IV than stream I (Table 38). Differences between the control and treated streams existed only in collections 7 and 8 when the Lemna from streams IV and III, respectively, had lower magnesium concentrations than the control. In conclusion, NTA exposure did not appear to affect the concentration of magnesium in Lemna.

PLANARIA

No Planaria were found in the copper treated stream (III) after treatment but for collection 7. NTA conferred protection from copper toxicity when added simultaneously in stream II, as samples were available throughout the experiment.

Copper (Fig. 34)

Differences between first and subsequent collections in:
Streams I, II. Copper concentration in Planaria showed no

(Control and NTA/Cu) significant differences between collection 1 and subsequent collections (Stream I, Kruskal-Wallis test; Stream II, Scheffe test).

Stream IV. (NTA) Concentrations in collections 2, 6, and 8 were significantly lower than in 1. There were no significant differences between any collections following NTA additions, suggesting that collection 1 was unusually high (Kruskal-Wallis test).

Differences between streams at each collection: Significant differences occurred prior to treatment (collection 1); Planaria from stream IV contained significantly more copper than those in streams I and II. There were no significant differences between the NTA treated streams and the control after treatments were initiated (Table 39).

Iron (Fig. 35)

Stream I. (Control) In collections 7 and 8, the Planaria had significantly lower iron concentrations than collection 1 (Kruskal-Wallis test).

Stream II. (NTA/Cu) Concentration in collection 8 was significantly lower than in collection 1 (Kruskal-Wallis test).

Stream IV. (NTA) No significant differences between first and subsequent collections existed (Scheffe test).

But for collections 4 and 8 there were no statistically significant differences in iron concentration in Planaria between streams (Table 39).

Zinc (Fig. 36)

Data for collection 5 were elevated for all streams, and although the coefficients of variation for the data from each stream were not unusually great, the data are excluded from analysis because of suspected contamination.

Differences between first and subsequent collections in stream:

Stream I. (Control) Concentrations in collections 3 and 8 were significantly lower than in collection 1 (Scheffe test).

Stream II. (NTA/Cu) No significant differences between collection 1 and subsequent collections existed, but variance in the data was great in collection 4 (Kruskal-Wallis test).

Stream IV. (NTA) No significant differences between the first and subsequent collections were found but variance in the data was great in collection 4 (Kruskal-Wallis test).

Differences between streams at each collection:
There was no significant difference between streams for zinc concentration in Planaria (Table 39). Lowered concentrations in algae exposed to NTA did not lead to lowered zinc concentrations in at least one animal that grazes on the algae.

Manganese (Fig. 37)

Differences between first and subsequent collections in stream:

Streams I, II, IV. (Control, NTA/Cu, and NTA)	There were no significant differences between the first and subsequent collections (Kruskal-Wallis test).

Differences between streams at each collection:
There were no significant differences between streams in manganese concentrations in Planaria at any collection time (Table 39).

Magnesium (Fig. 38)

Differences between first and subsequent collections in streams:

Streams I, II. (Control, NTA/Cu)	Collections 5 and 6 were significantly higher than collection 1 (Scheffe test).
Stream IV. (NTA)	There were no significant differences between the first and subsequent collections (Kruskal-Wallis test).

Differences between streams at each collection:
There were no significant differences in magnesium concentration in Planaria between streams at any collection except collection 4 when the concentration in stream II material was significantly higher than in stream I material, and in collection 6 when the concentration in Planaria from stream IV was significantly lower than in stream I material (Table 39).

Samples of tubificid worms were taken from the sediments of each stream for metal analyses at collections 3-8. Most times only a single sample of sufficient dry weight for metal analysis could be obtained with the sampling effort possible. Therefore, the data from all collections has been analyzed together. Copper was the only metal for which statistically significant differences between streams was obtained (Table 40). Mean concentrations in animals from the streams receiving copper (II and III) were significantly greater than in samples from the control but differences between the treated streams were not significant.

In another analysis, concentration factors were calculated for each sample type to assess bioaccumulation. For each metal, concentration in the biomass from a given stream was divided by the cumulative mean concentration in the water of that stream to that point in time ($\mu g/g \times 1000 \div \mu g/l$, or ppb ÷ ppb). These concentration factors were calculated for each collection from 4-8 and the mean was obtained. Concentrations of metals in the water are shown in Table 41. Where measured concentrations were below the lower detection limit of the analysis procedure, the lower detection limit was used as the concentration in calculating the cumulative mean. Concentration factors and the results of statistical tests for significant differences are summarized in Table 42. Variance in the data was large and no significant differences between the control stream (I) and any of the experimental streams were obtained. Usually all streams grouped together (no significant differences in concentration factor). In the two instances when they did not (algae, Cu and Planaria, Cu) the samples from control still grouped with those from each NTA stream suggesting no effect from NTA exposure on metal accumulation.

The possible biomagnification of metals between Planaria and algae was examined also (Table 43). Only zinc showed biomagnification in the Planaria tissue; the other metals showed reduced concentrations in the animal tissue compared to the plant tissue. For zinc there was a significant difference between streams; the ratio was greater in streams containing NTA than in the control.

Although samples of sediments were cored from each stream for metal analyses, the data suggested that after the first few collections the sediments were mixed, which probably occurred as samples were removed. The sampling area was limited by the size of pools in each stream and so samples could not be obtained from undisturbed areas for the entire experiment. Therefore, another experiment was performed in recirculating microcosms to simulate exposure of sediments in a flowing water environment to copper and NTA over a period of 9 weeks. NTA and copper were added to provide 30 μg Cu^{++}/l and 2.0 mg NTA/l in the experimental microcosms (Table 44).

Concentrations of copper were measured at the sediment surface and at 2 and 5 cm depths four times through the experiment (Table 45). A mean copper concentration was calculated for the experiment using the mean of each of the four sampling times and between treatments and depths. Copper concentrations in the control did not differ with depth but in sediments exposed to Cu or Cu/NTA, the copper concentration at the surface was significantly higher than at 5 cm but not 2 cm. There were no statistically significant differences in copper concentration as a result of treatment at the 2 and 5 cm depths. At the surface concentration was greater in the Cu/NTA and Cu only treatments than in the control. However, only the Cu/NTA treatment differed significantly from the control and it did not differ significantly from the copper treatment. Sanchez and Lee (58), however, noted that NTA enhanced the adsorption of copper by lake sediments.

At the end of the experiment sediments were also tested for concentrations of iron, manganese and zinc (Table 46). These did not differ significantly with depth in any treatment (Kruskal-Wallis test, $P = 0.05$ for Zn in control and copper treatments otherwise Scheffe tests, $P = 0.05$). There were no statistically significant differences between treatments for manganese and zinc concentration at any depth or for iron at the surface and 5 cm. At the 2 cm depth, however, iron concentration was inexplicably high in the Cu and Cu/NTA exposed sediments. Note that zinc concentrations were near the lower detection limit of the method used for analysis.

CONCLUSIONS

NTA clearly protected the organisms in stream II from the toxic effects of copper. Copper concentrations in algae, *Anacharis*, *Lemna* or *Planaria*, from stream II were not elevated over control data when copper was added as the NTA chelate. Zinc concentrations in samples of algae, *Anacharis*, and *Lemna* exposed to NTA were lower than concentrations in samples from the control stream in several collections. Sufficient NTA (2 mg/l, 10^{-5} $\underline{M}$) to completely chelate the copper (30 μg/l, 4×10^{-7} $\underline{M}$) was used which also would have complexed with some (if not all) the zinc(1×10^{-6} $\underline{M}$) present in the White Clay Creek water in stream II. Where NTA was added alone (stream IV) the probability of zinc-NTA complex formation was all the greater. It is likely that the lower concentrations in the biota resulted from the chelation of zinc by NTA and relative inaccessability of the NTA-zinc complex to the biota compared to the zinc-carbonate complex which predominated in the control (Chance, unpublished M.S. thesis, University of Pennsylvania, has studied the chemistry of inorganic ions in White Clay Creek water). Examination of concentration factors lends support to this observation. For zinc, these were lower in the NTA containing streams than those not containing NTA (although statistical significance was not noted). Concentration of magnesium, manganese and iron were probably not affected by the presence of NTA although iron in *Anacharis* may have been lower owing to the presence of NTA and should be more thoroughly studied in the future work with the compound.

Although 2 mg/l NTA protected the biota from harmful effects of 30 μg/l copper, copper accumulation in sediments was not prevented by NTA at this level nor did depletion through extraction occur. Similarly, the accumulation of Cu in sediment dwelling worms was also unaffected. Copper concentrations in tubificids from both streams to which copper was added were elevated over the values for the control stream even when NTA was added simultaneously.

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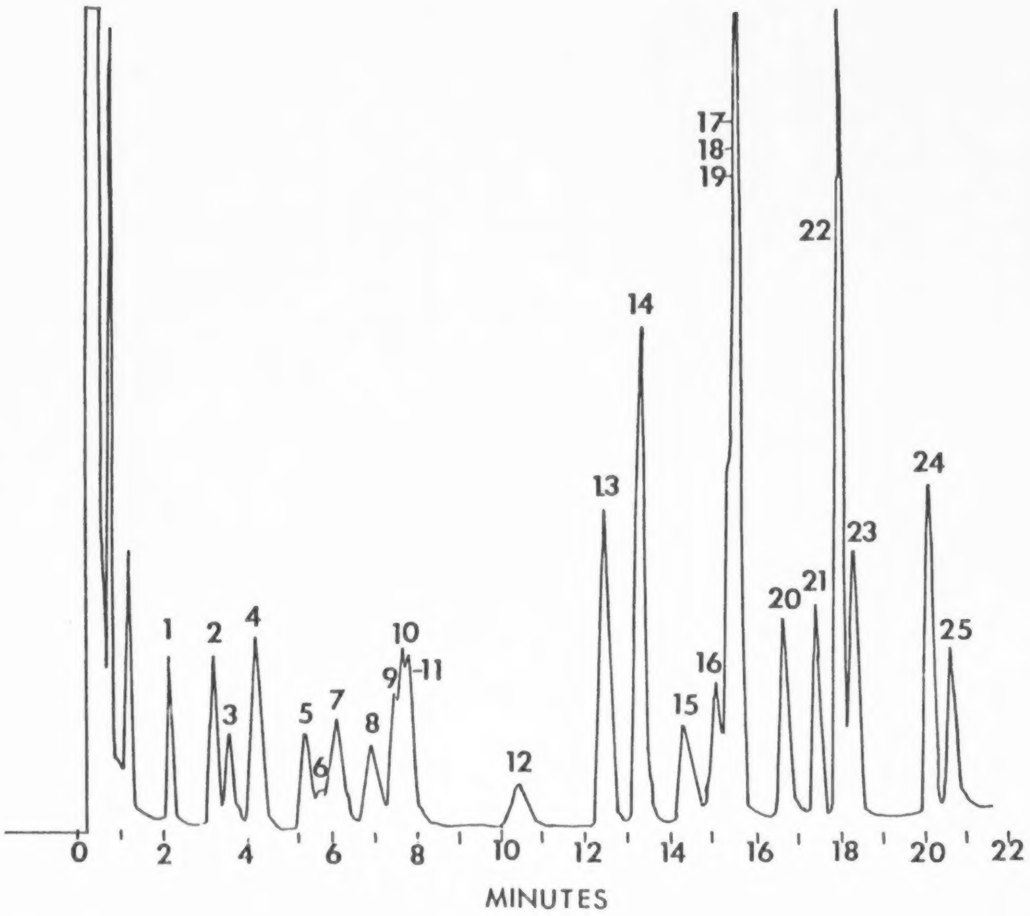

Figure 4. GC separation of butyl ester of twenty-five carboxylic acids. Numbers refer to acids in Table 1. (Reproduced by permission from J. Chromatography.)

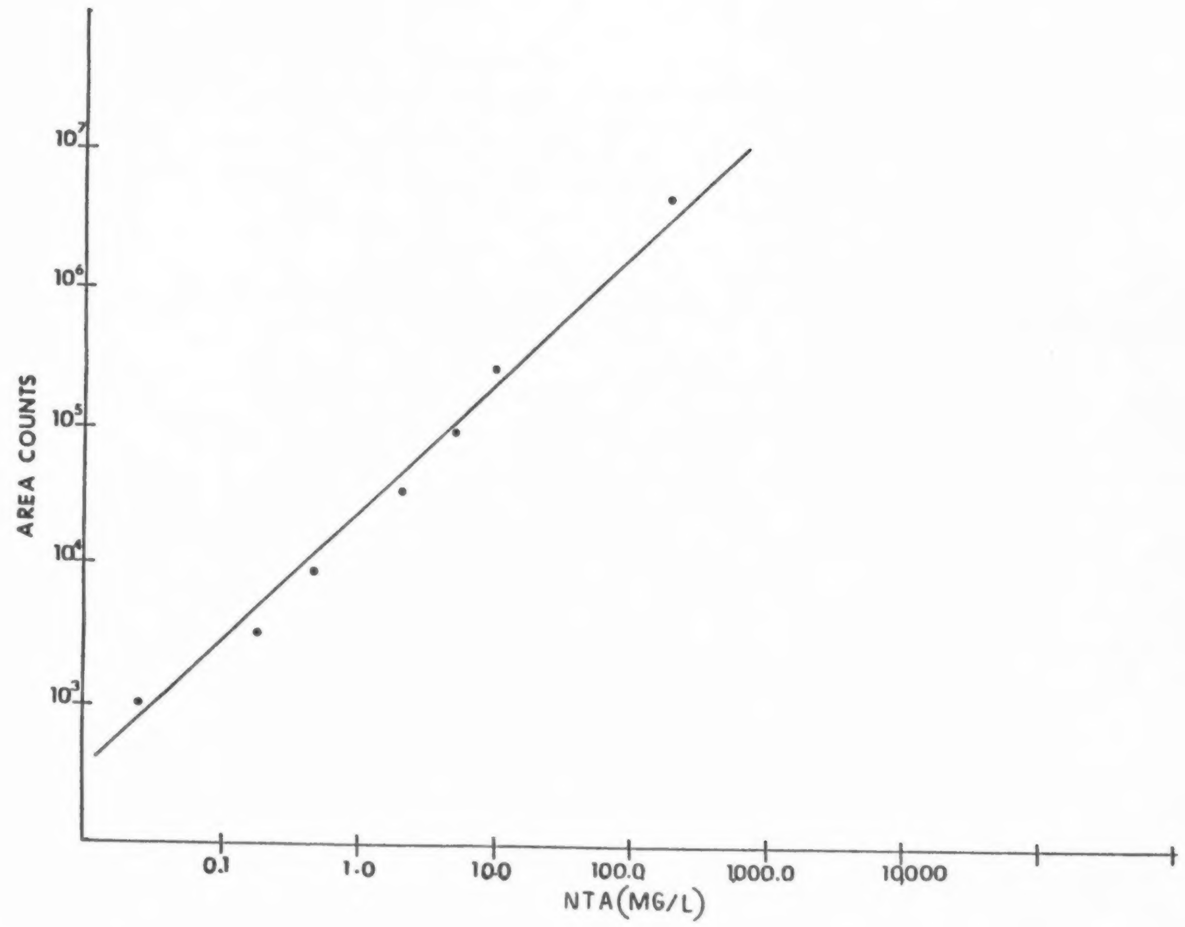

Figure 5. Standard curve for NTA gas chromatographic analysis. (Reproduced by permission from _J. Chromatography_.)

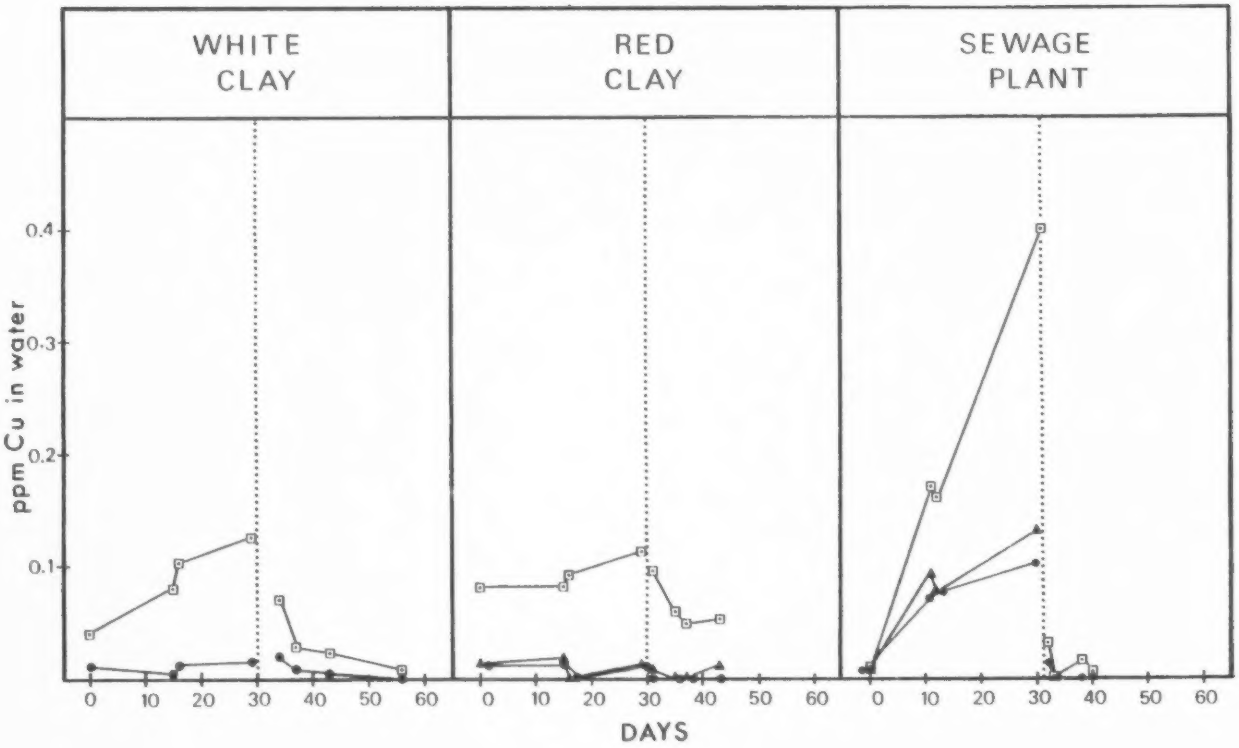

Figure 6. Effect of NTA on solubilization of copper from three stream sediments. Squares, 10^{-3} M (191 mg/l) NTA: diamonds, 10^{-5} M (1.91 mg/l): circles, controls. Vertical dotted line indicates end of sterile portion of experiment.

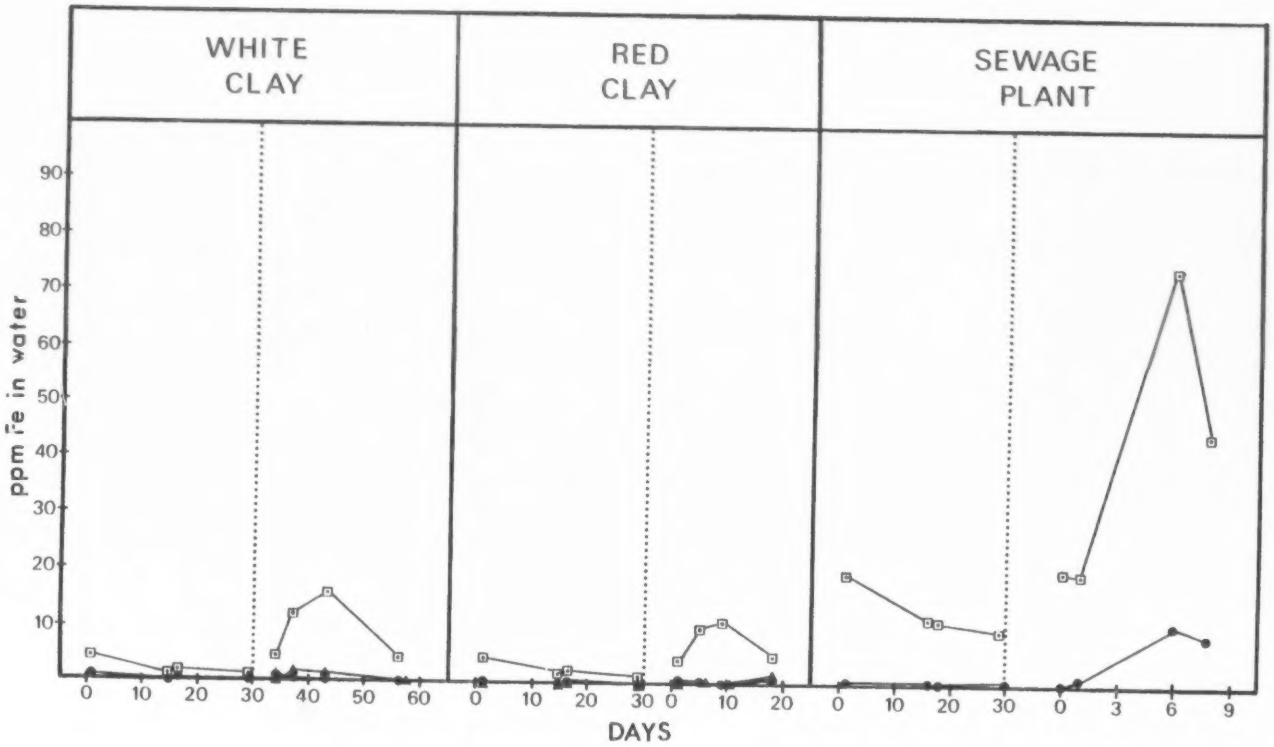

Figure 7. Effect of NTA on solubilization of iron from three stream sediments. Symbols same as in Figure 6 except that diamonds also include data for 10^{-7} M (0.02 mg/l) NTA.

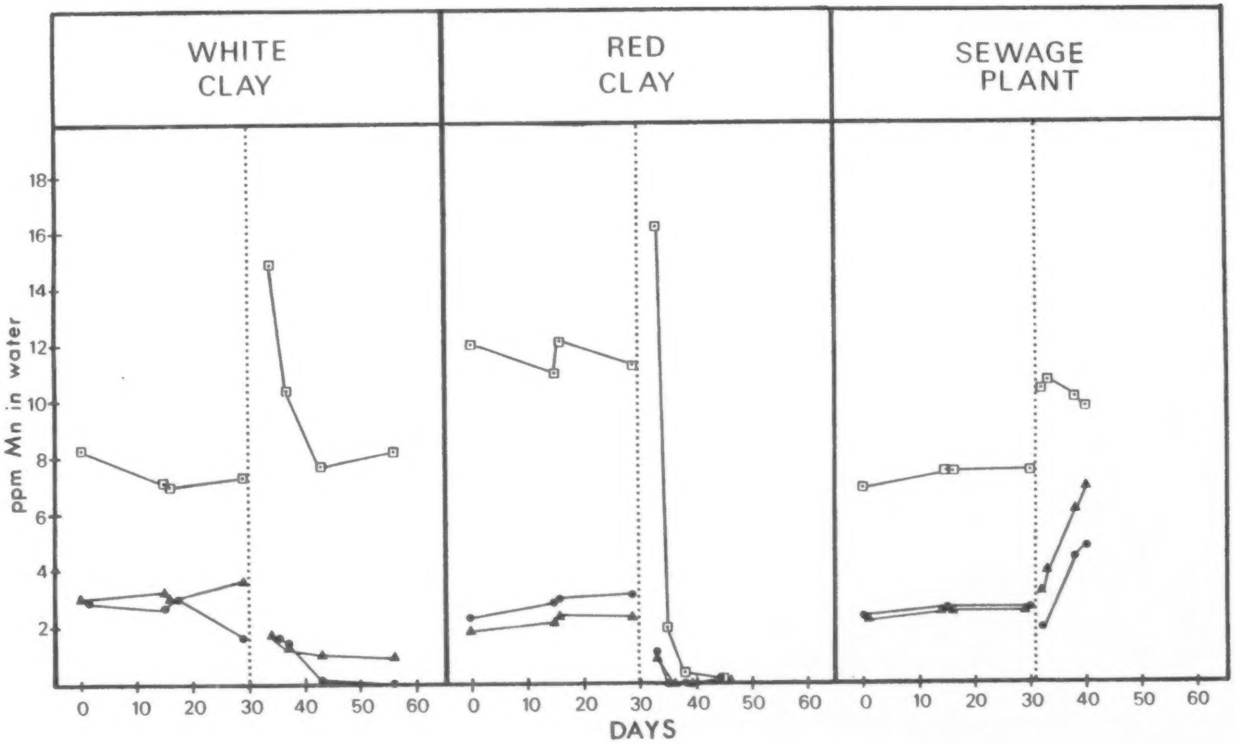

Figure 8. Effect of NTA on solubilization of manganese from three stream sediments. Symbols same as in Figure 7.

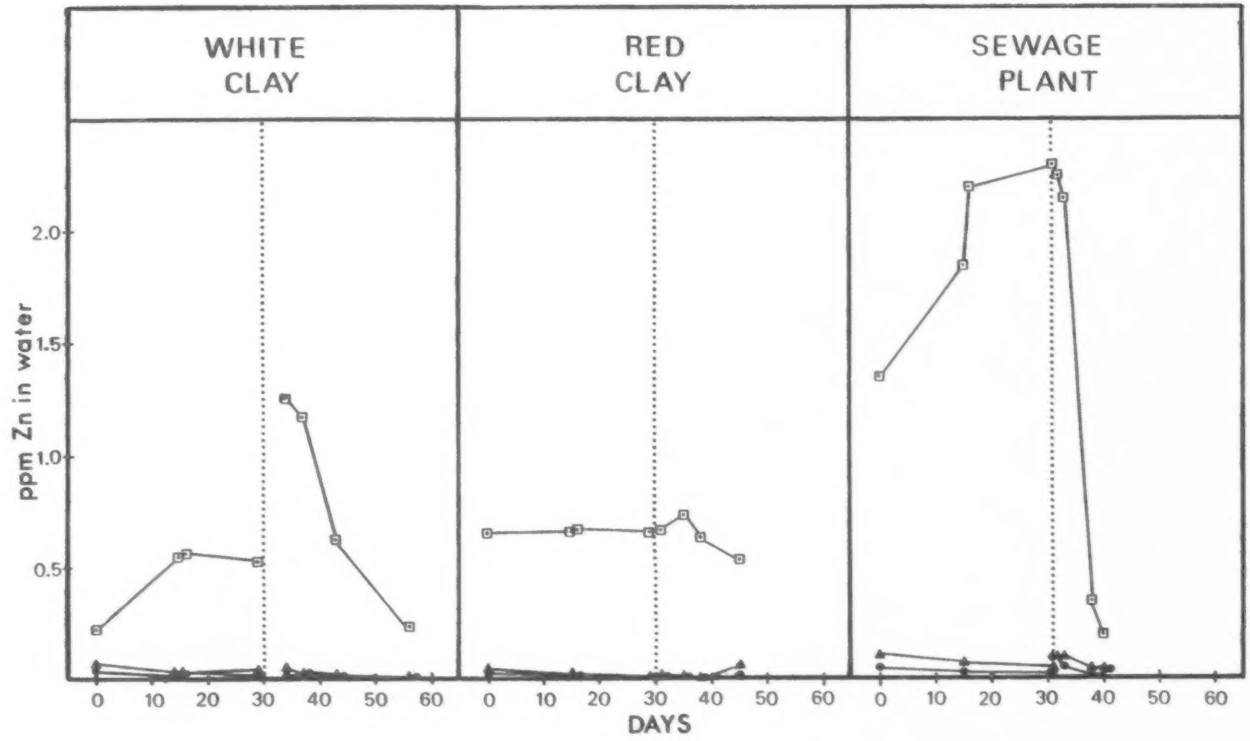

Figure 9. Effect of NTA on solubilization of zinc from three stream sediments. Symbols same as in Figure 7.

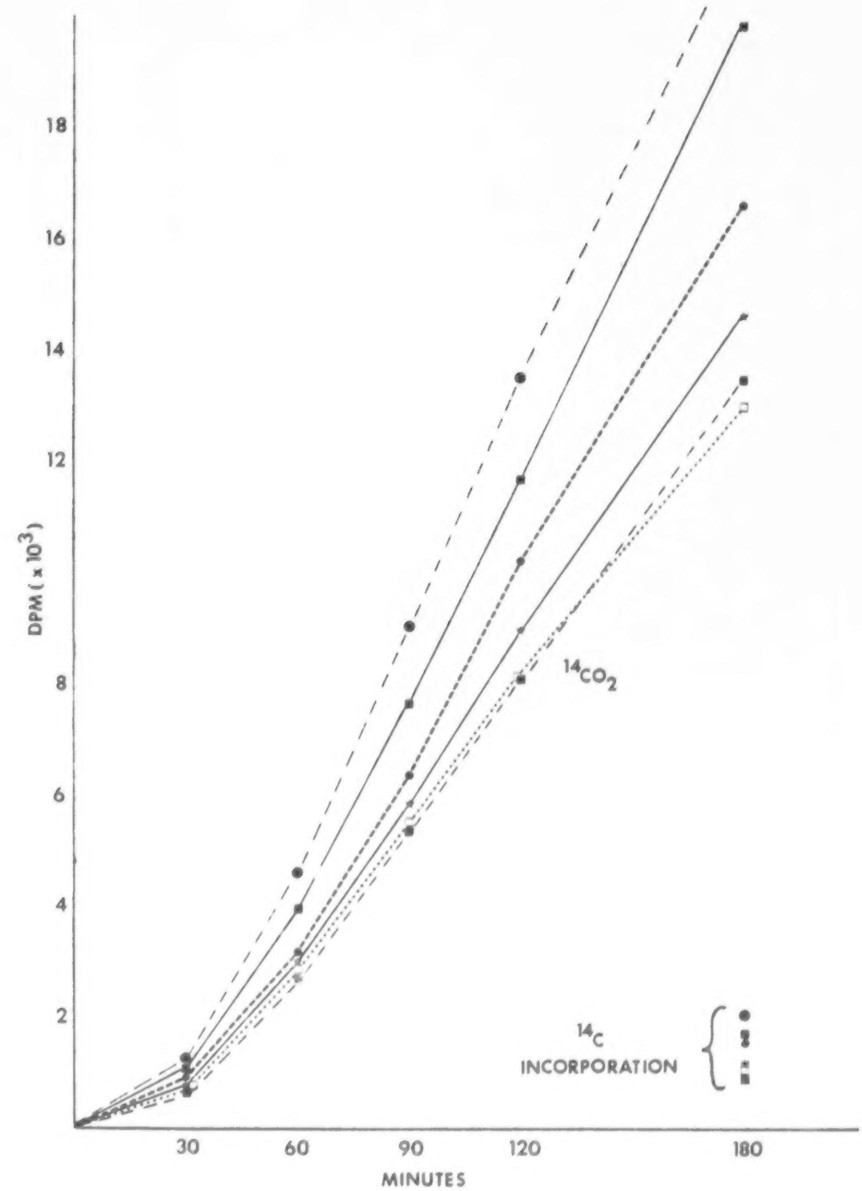

Figure 10. Mineralization ($^{14}CO_2$ evolution) of NTA by cell suspensions of a Pseudomonas sp. known to degrade NTA. ^{14}C incorporation by cells also shown.

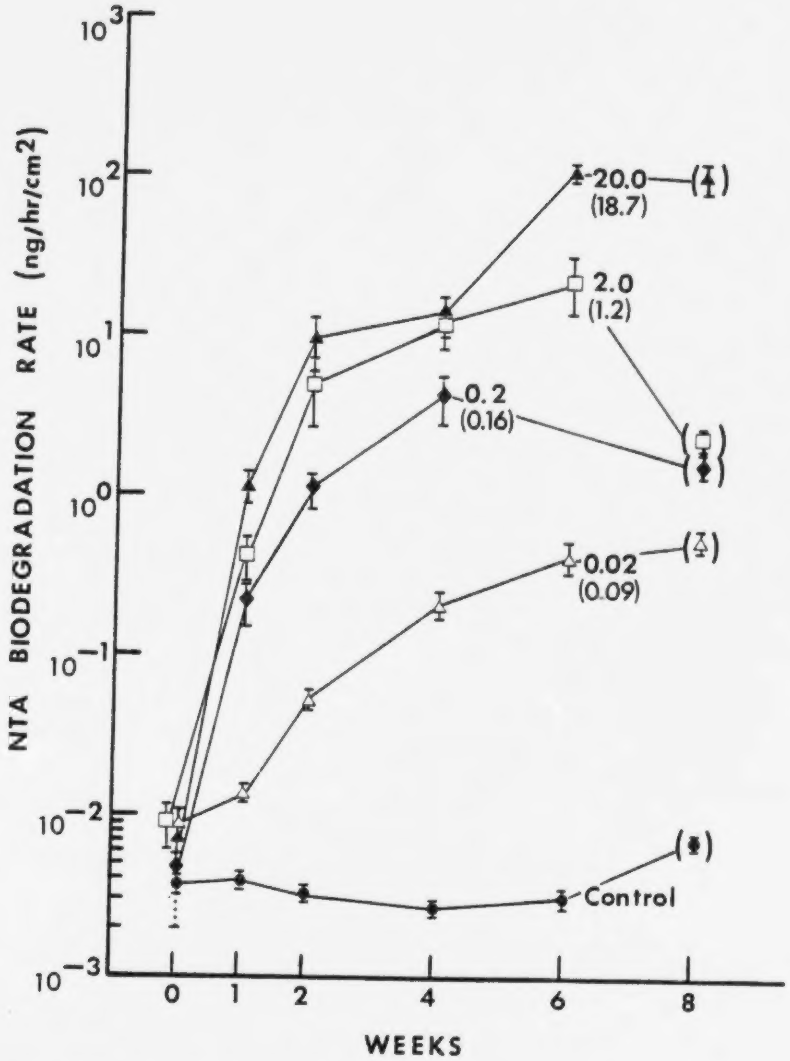

Figure 11. Semi-log plot of NTA degradation rate ($\overline{x} + $ s.d.) by bacterial populations at 9°C. (March-April, 1975). Data at 8 weeks indicated parenthetically because of temperature rise to 12°C in microcosms. Mean NTA exposure indicated in parenthesis along with desired exposure in mg/l.

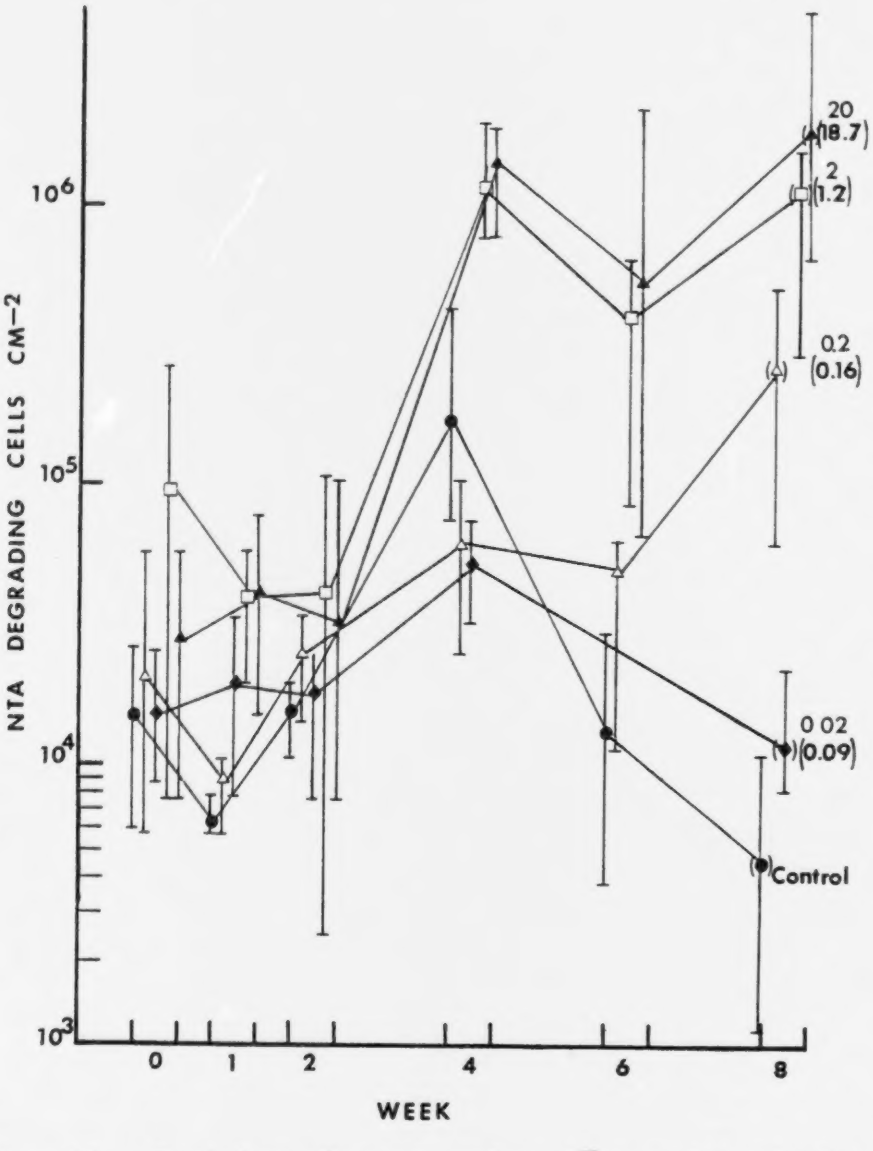

Figure 12. Semi-log plot of number ($\bar{x}$ ± s.d.) of NTA degrading bacteria on coverslips exposed to NTA at the indicated concentrations (March-April 1975). NTA exposure shown as in Figure 11. Data for 8 week sampling indicated parenthetically as in Figure 11.

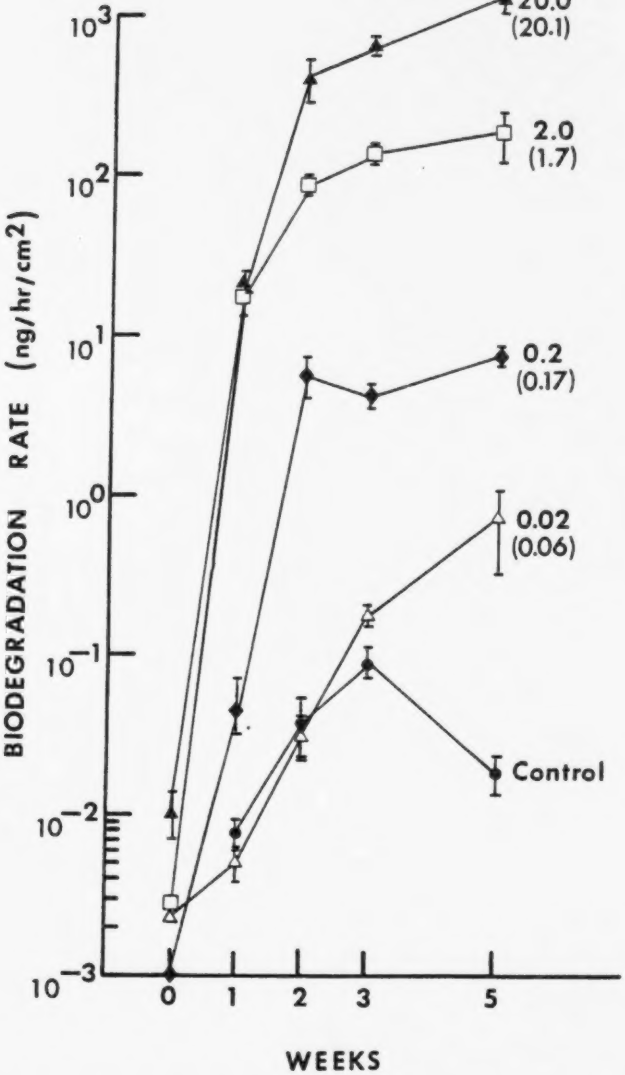

Figure 13. Semi-log plot of NTA degradation rate ($\bar{x} \pm$ s.d.) by bacterial populations at 18°C (July-August, 1975). Actual concentration of exposure shown in parenthesis along with desired exposure in mg/l.

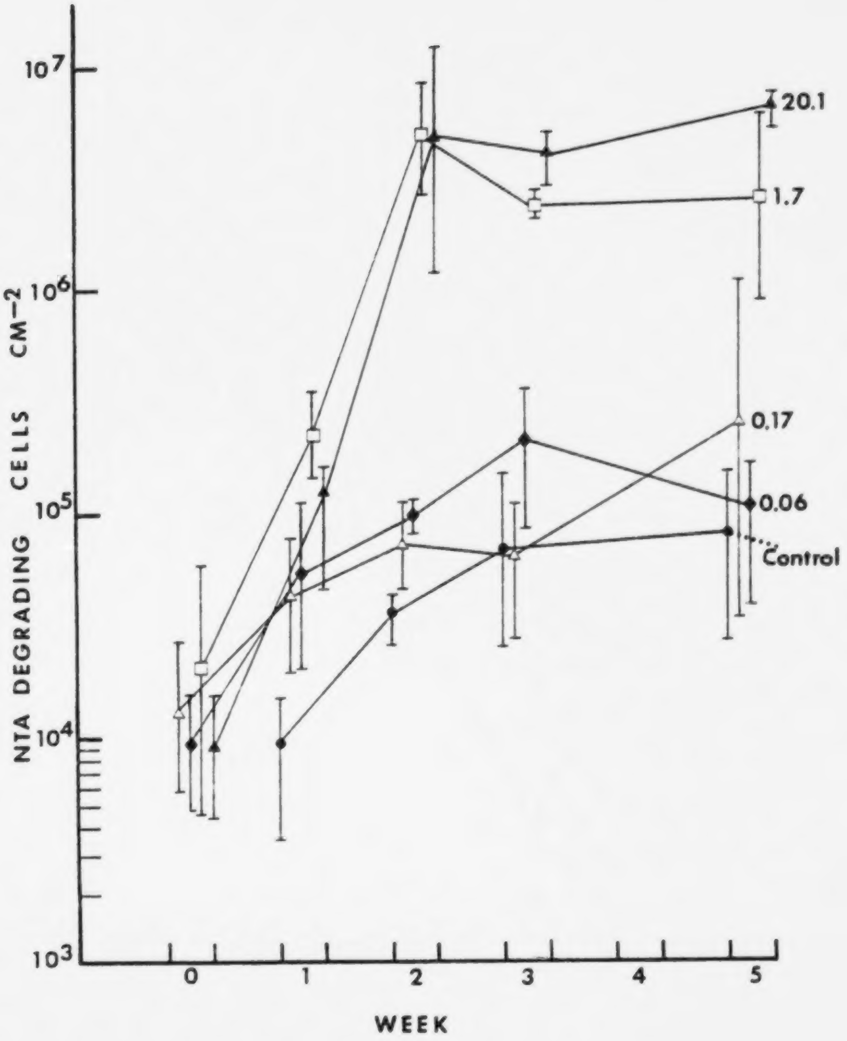

Figure 14. Semi-log plot of number of NTA degrading bacteria ($\bar{x} \pm$ s.d.) on coverslips exposed to NTA at approximately the indicated concentrations.

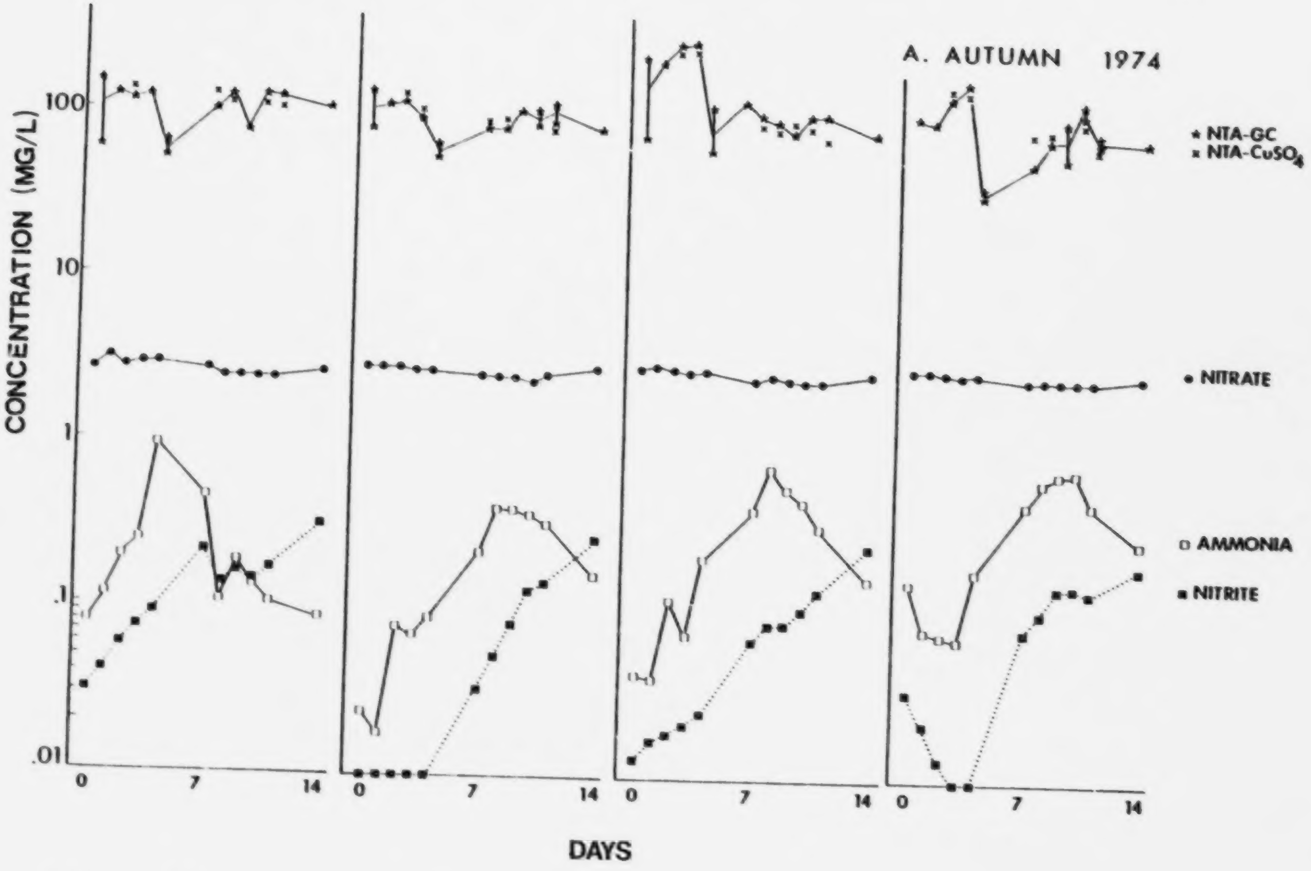

Figure 15. A, B, C. Changing concentrations of NH_3-N, NO_2-N, and NO_3-N resulting from the degradation of NTA in 87 l microcosms. Note concentrations placed on log scale to facilitate comparison of rates of appearance and disappearance of components in widely differing concentration ranges.

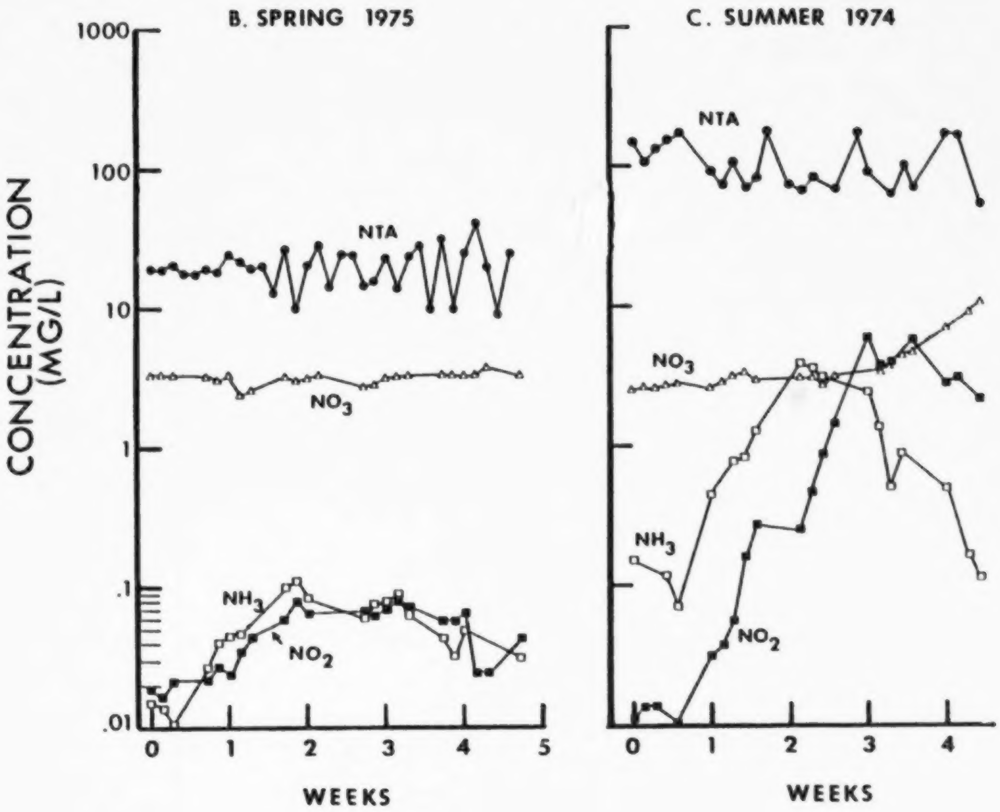

Figure 15. B, C. (Continued)

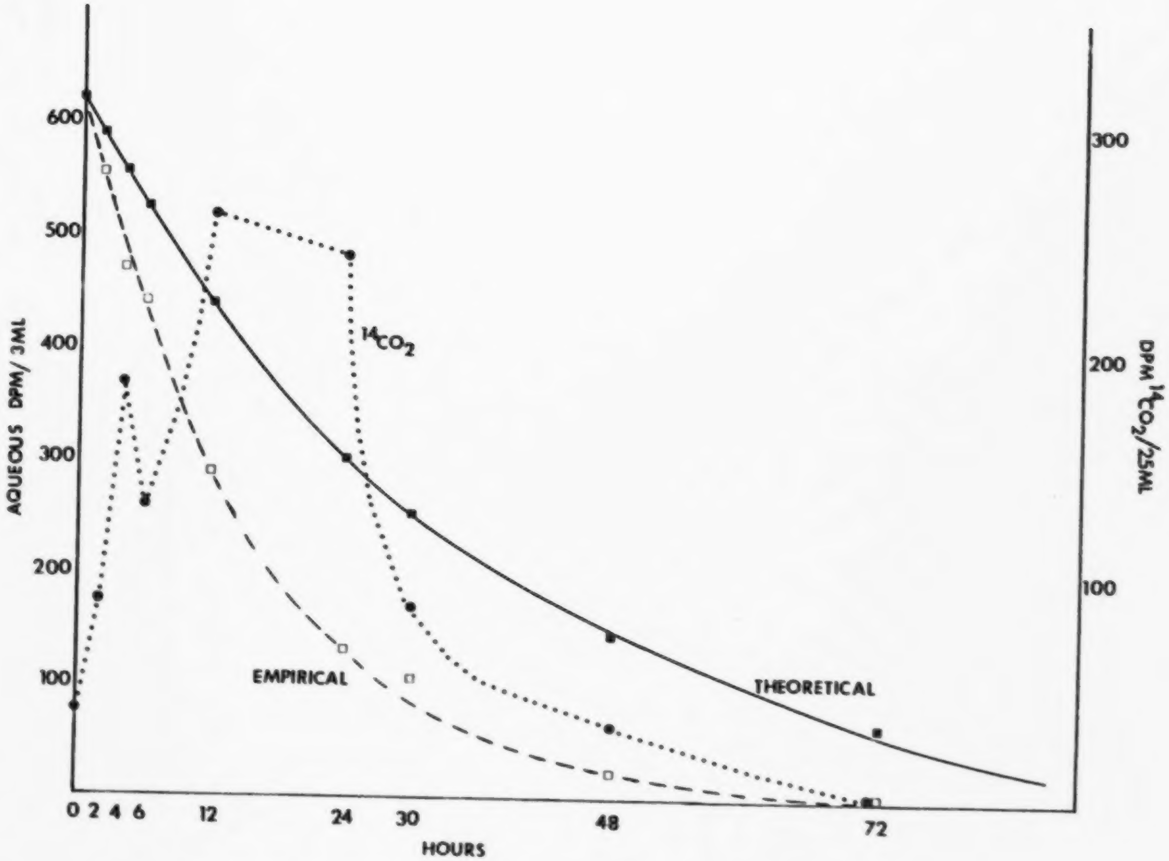

Figure 16. Changing concentrations of ^{14}C-NTA in an 87 l microcosm system resulting from dilution (Theoretical curve) and both dilution and bacterial decomposition (Empirical curve) (December, 1974).

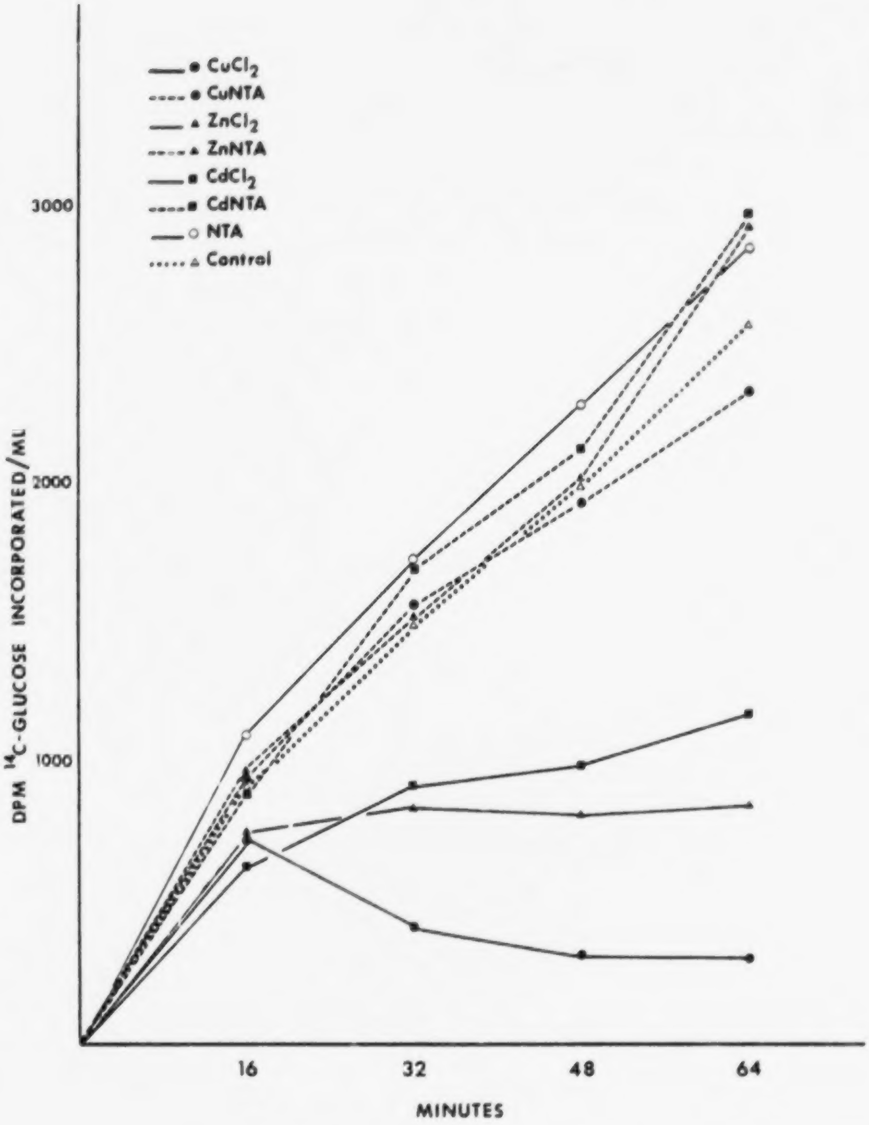

Figure 17. Incorporation of ^{14}C glucose by washed cells from a culture of naturally occurring bacteria in the presence of added metal ions or NTA-metal chelates. NTA probably present as the Ca- and Fe-chelate when added alone. Assay performed at 9°C.

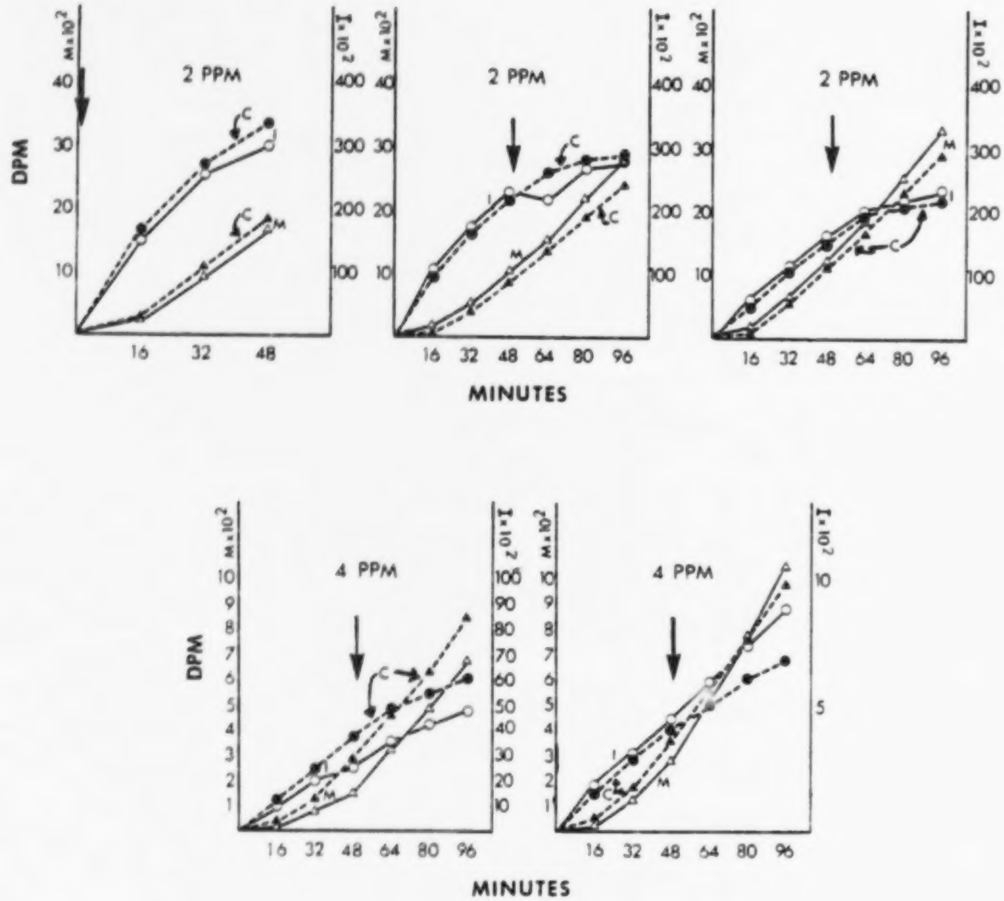

Figure 18. Incorporation (I = O ●, open = NTA exposed, closed = control) and mineralization (M = Δ ▲, open = NTA exposed, closed = control) of ^{14}C glucose by suspensions of washed cells harvested from cultures of mixed natural populations. NTA (0.25) ml added at arrow to provide indicated concentrations. Controls inoculated with an equivalent volume of water.

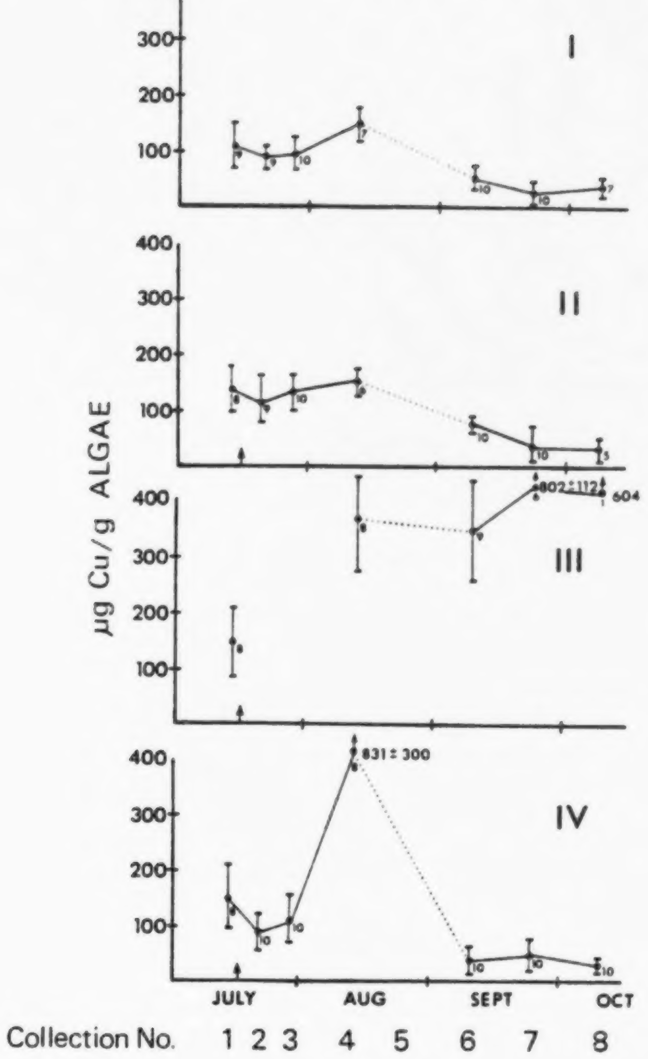

Figure 19. Concentration of copper ($\bar{x} \pm$ s.d.) in algal communities from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

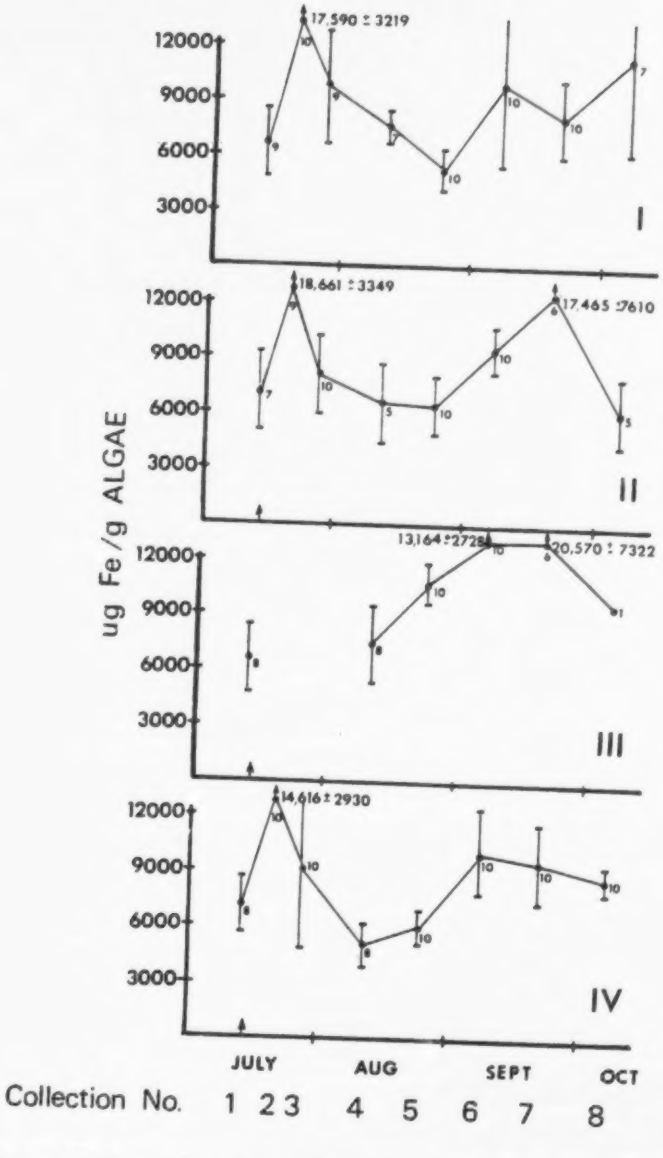

Figure 20. Concentration of iron ($\bar{x} \pm$ s.d.) in algal communities from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

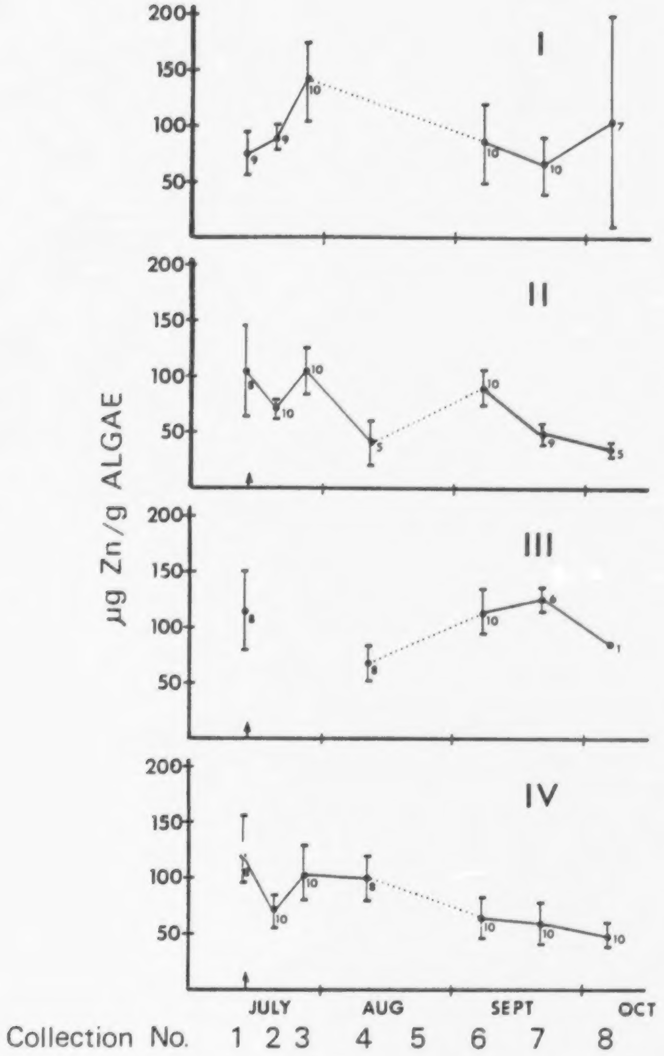

Figure 21. Concentration of zinc ($\bar{x} \pm$ s.d.) in algal communities from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size.

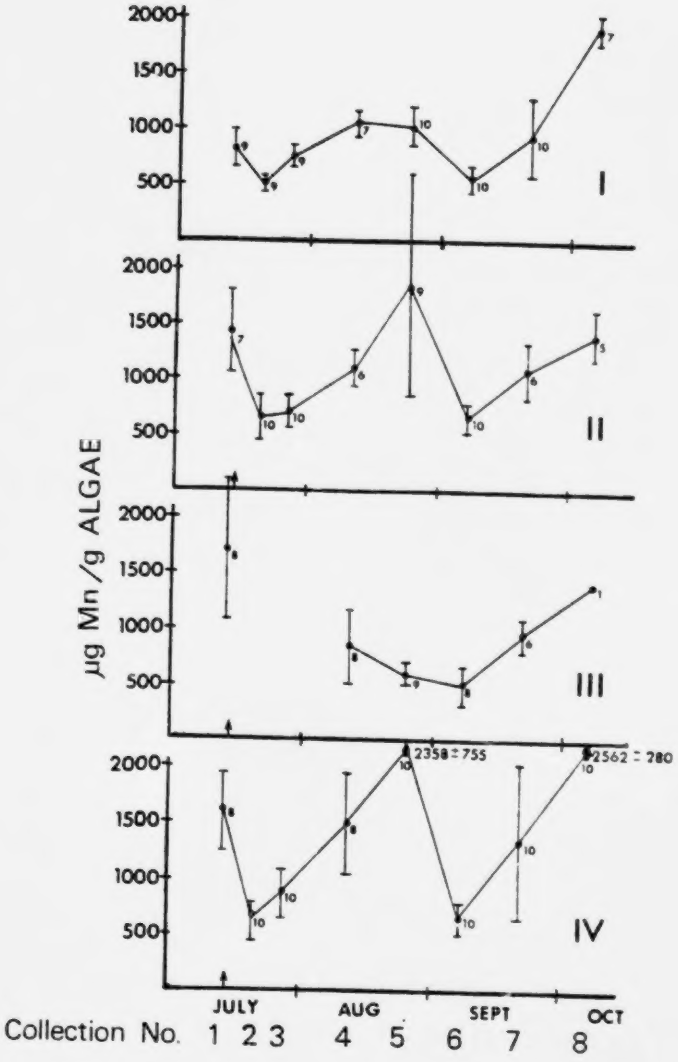

Figure 22. Concentration of manganese ($\bar{x} \pm$ s.d.) in algal communities from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

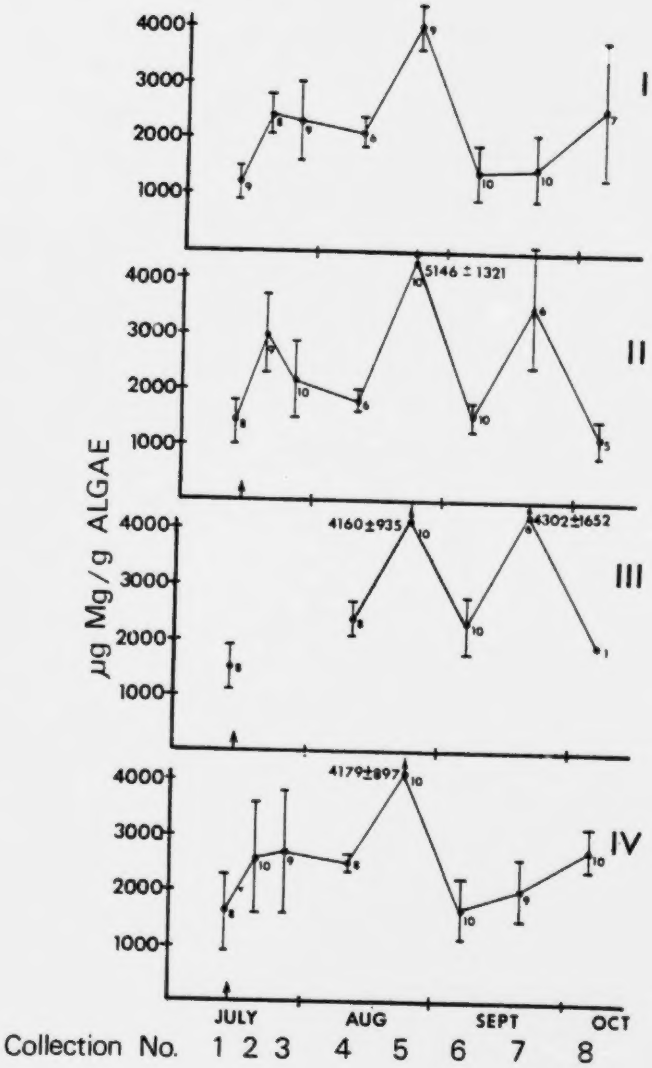

Figure 23. Concentration of magnesium ($\bar{x}$ ± s.d.) in algal communities from ecosystem streams through time. Stream I-Control, II-NTA, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate x ± s.d. of data off-scale.

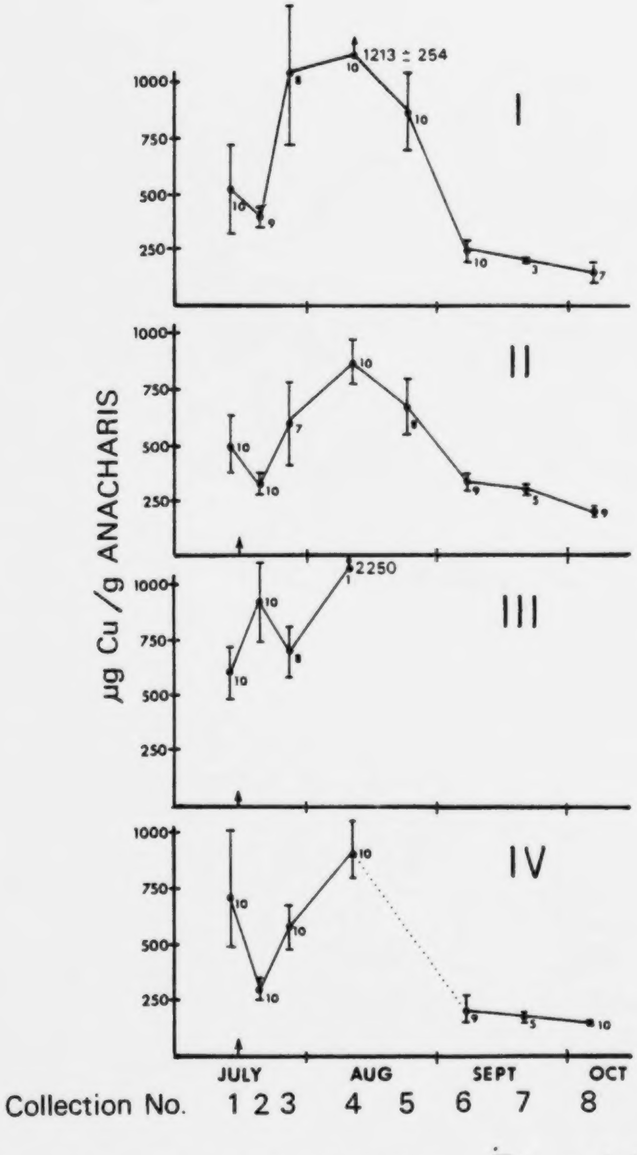

Figure 24. Concentration of copper ($\bar{x} \pm$ s.d.) in Anacharis from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

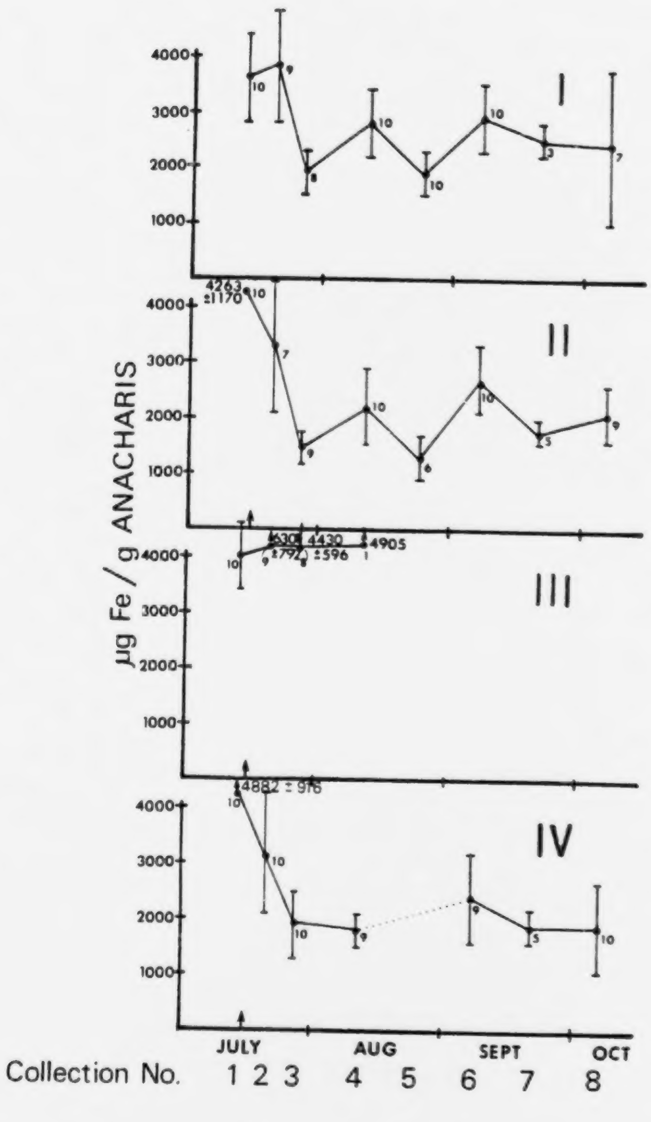

Figure 25. Concentration of iron ($\overline{x} \pm$ s.d.) in Anacharis from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\overline{x} \pm$ s.d. of data off-scale.

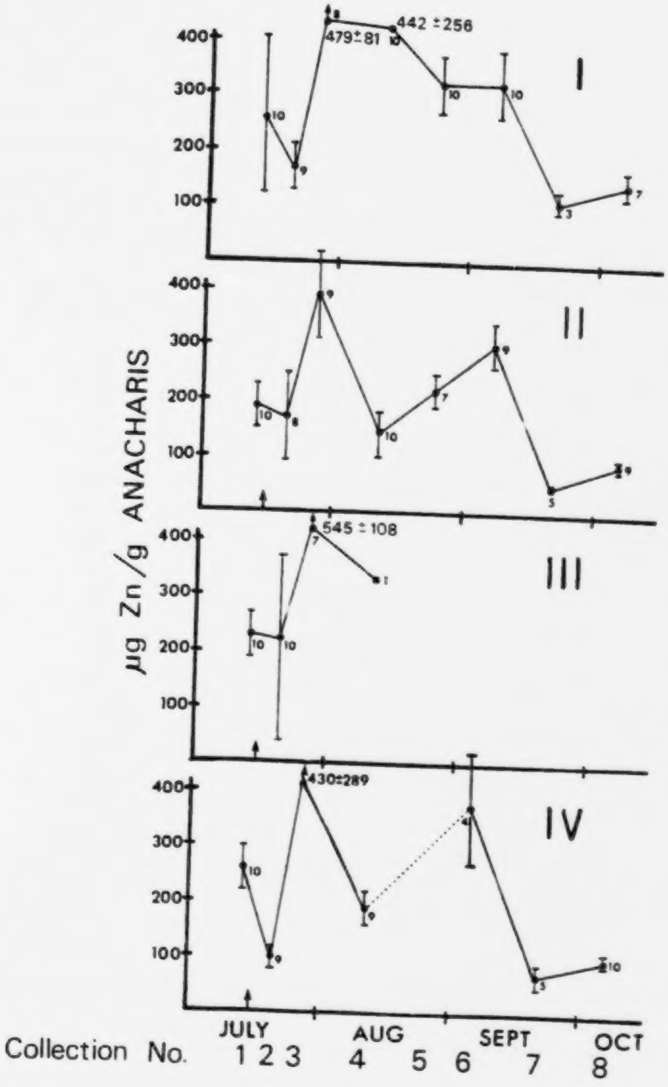

Figure 26. Concentration of zinc ($\bar{x} \pm$ s.d.) in Anacharis from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

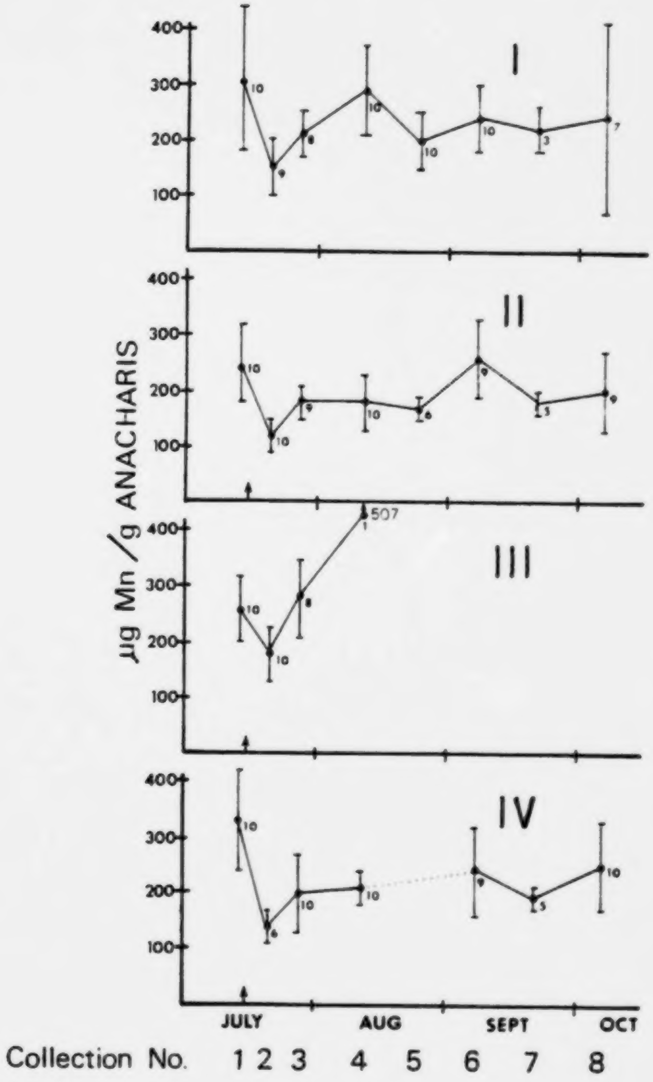

Figure 27. Concentration of manganese ($\bar{x} \pm$ s.d.) in Anacharis from ecosystem streams through time. Stream I - Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

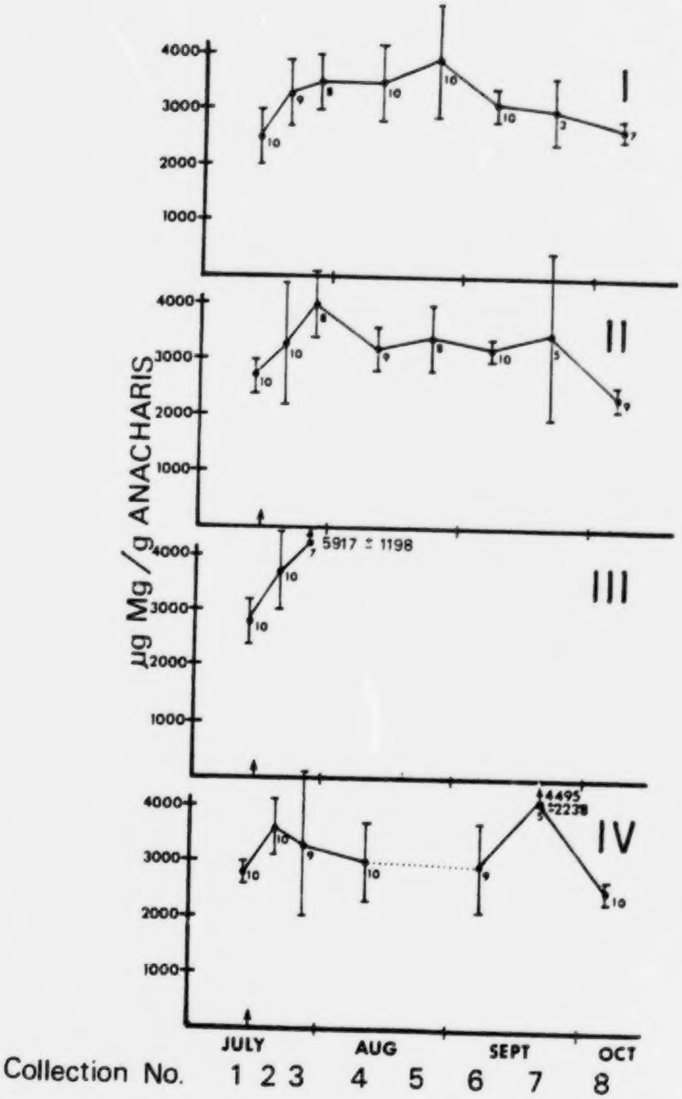

Figure 28. Concentration of magnesium ($\bar{x} \pm$ s.d.) in *Anacharis* from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

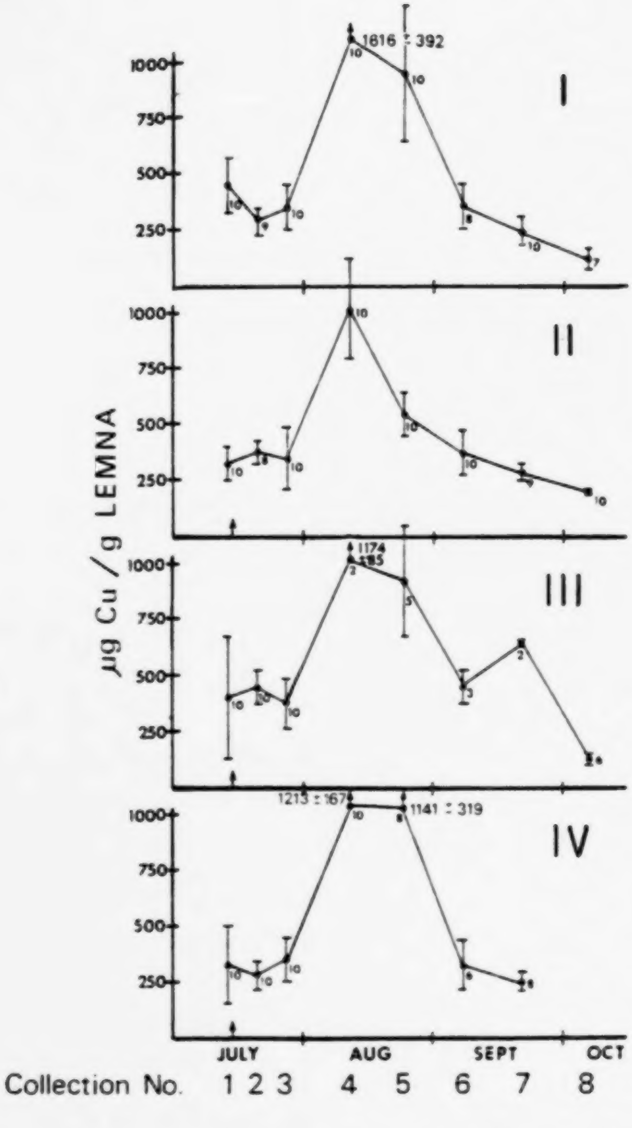

Figure 29. Concentration of copper ($\bar{x} \pm$ s.d.) in Lemna from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

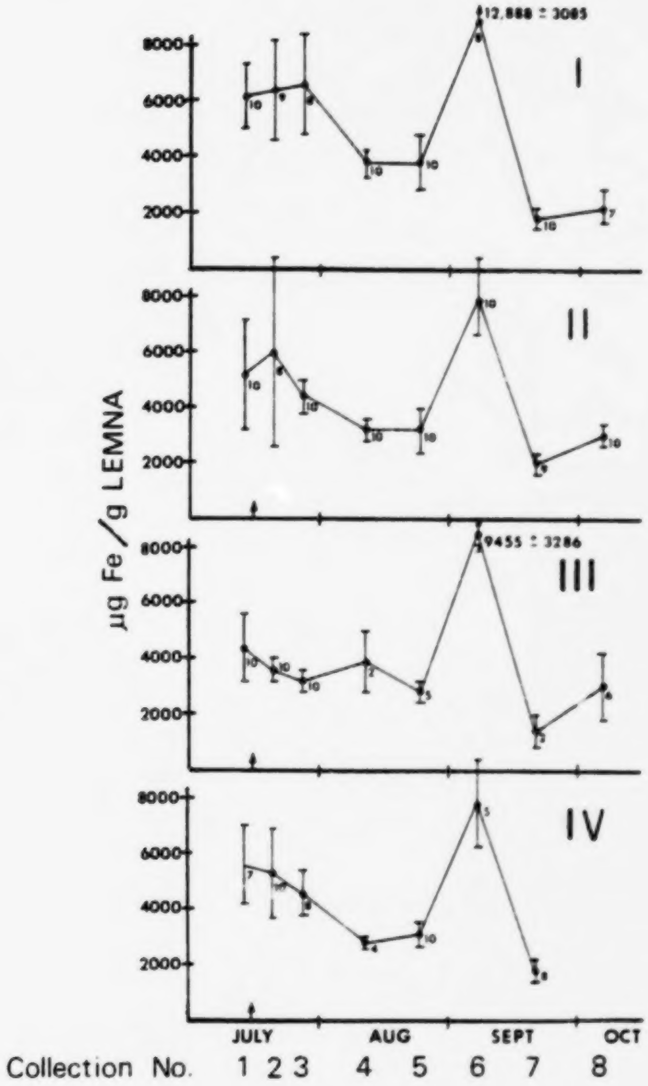

Figure 30. Concentration of iron ($\bar{x} \pm$ s.d.) in Lemna from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

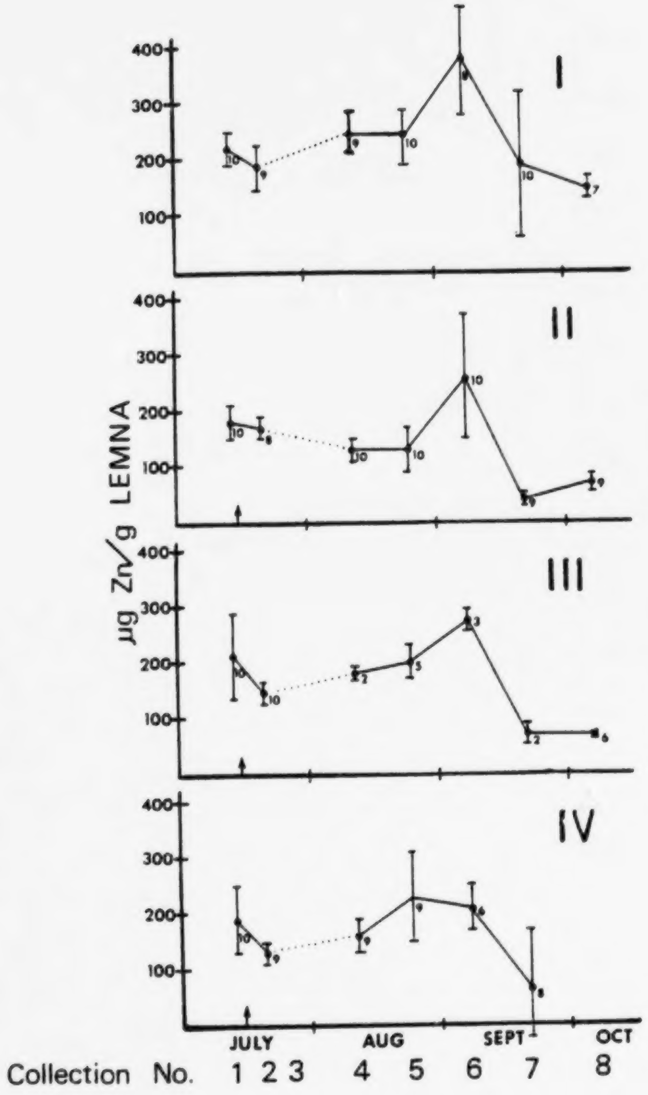

Figure 31. Concentration of zinc ($\bar{x} \pm$ s.d.) in Lemna from ecosystem streams through time. Stream I-control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size.

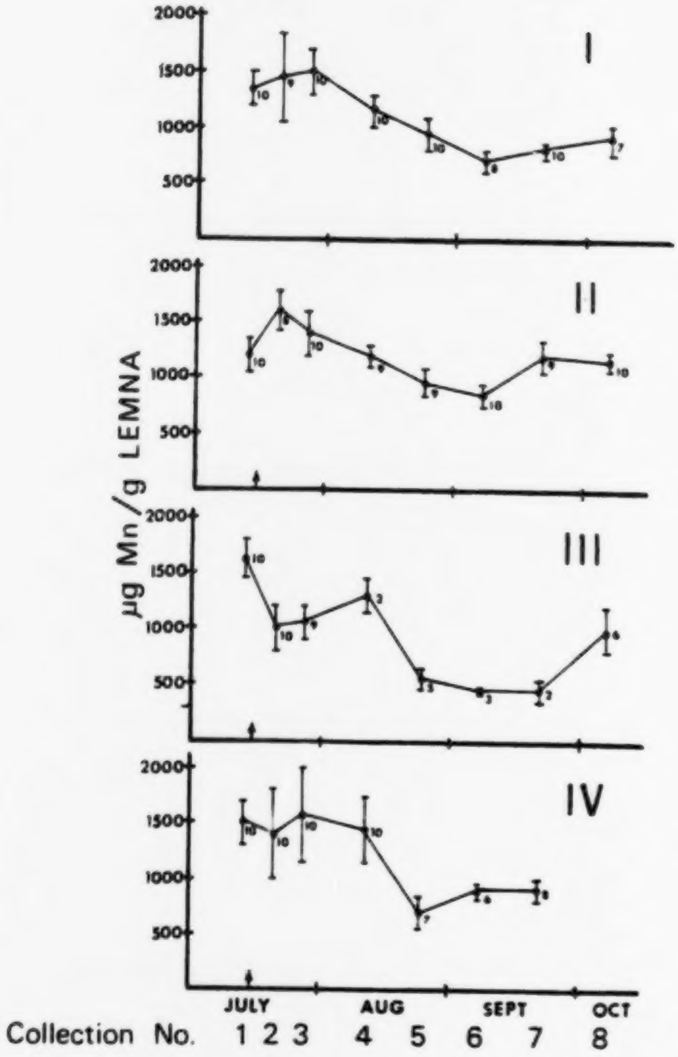

Figure 32. Concentration of manganese ($\bar{x} \pm$ s.d.) in *Lemna* from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size.

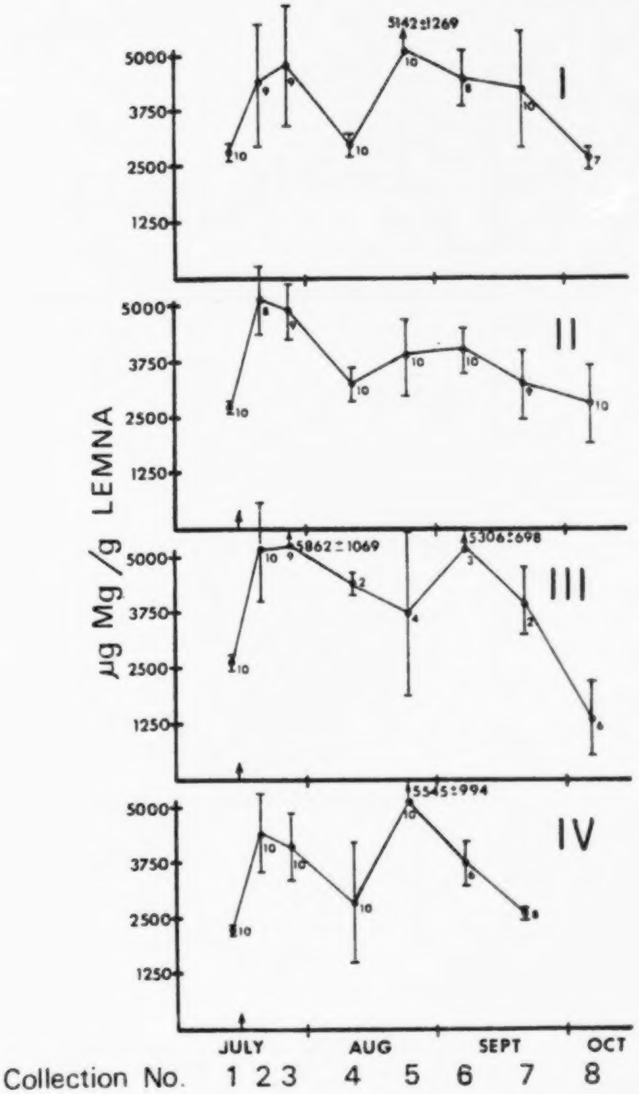

Figure 33. Concentration of magnesium ($\bar{x} \pm$ s.d.) in Lemna from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

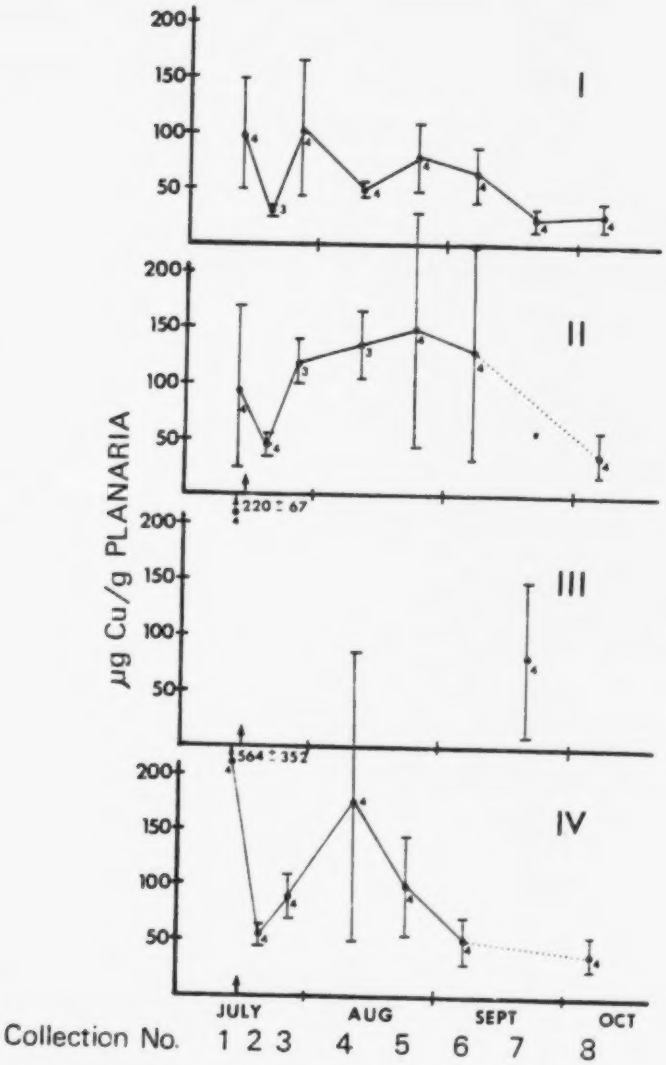

Figure 34. Concentration of copper ($\bar{x} \pm$ s.d.) in Planaria from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

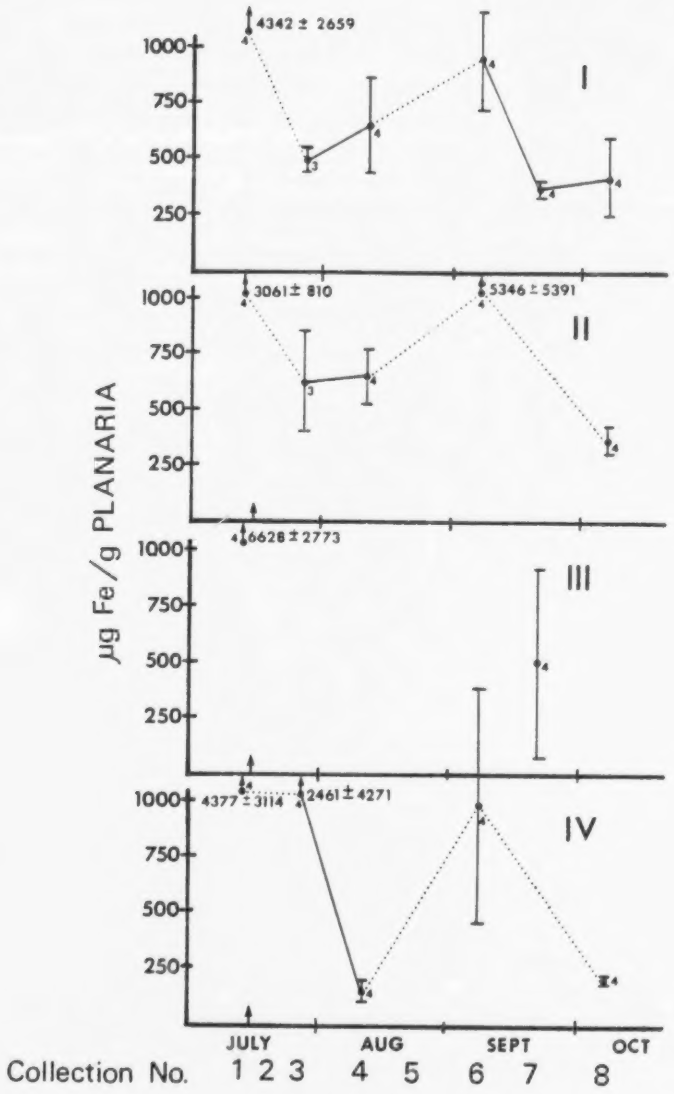

Figure 35. Concentration of iron ($\bar{x} \pm$ s.d.) in Planaria from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

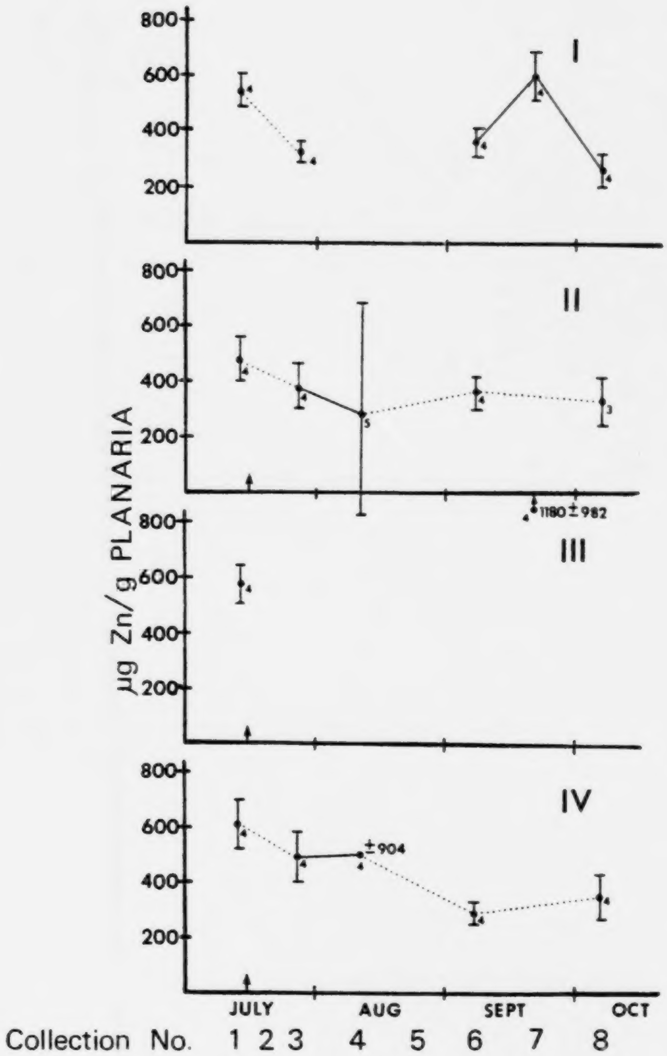

Figure 36. Concentration of zinc ($\bar{x} \pm$ s.d.) in Planaria from ecosystem streams through time. Stream I-control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size. Larger numbers indicate $\bar{x} \pm$ s.d. of data off-scale.

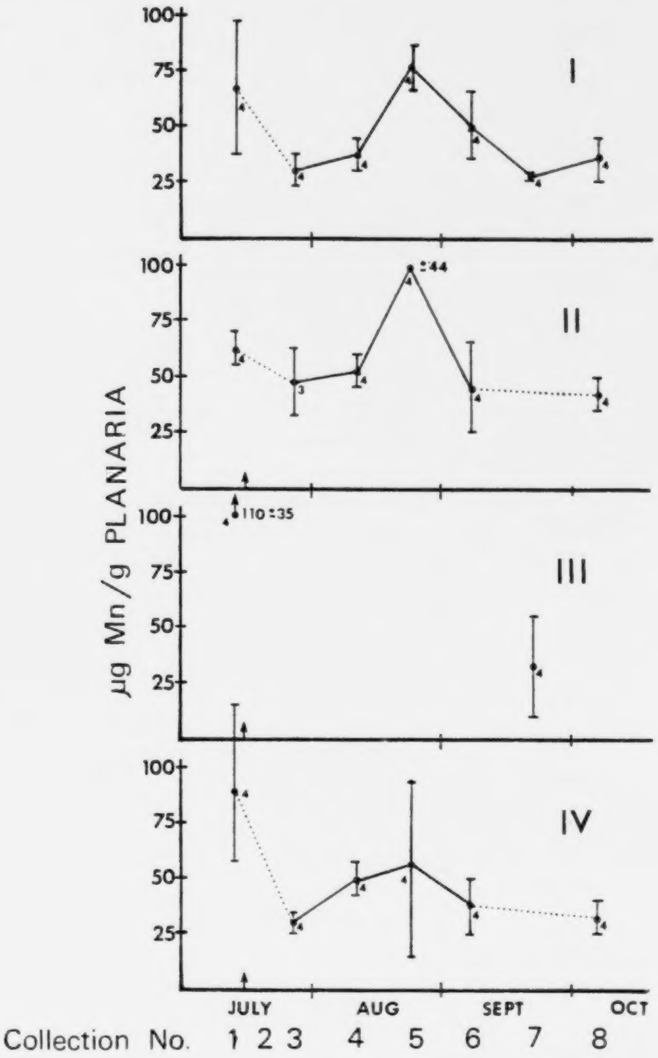

Figure 37. Concentration of manganese ($\bar{x} \pm$ s.d.) in *Planaria* from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size.

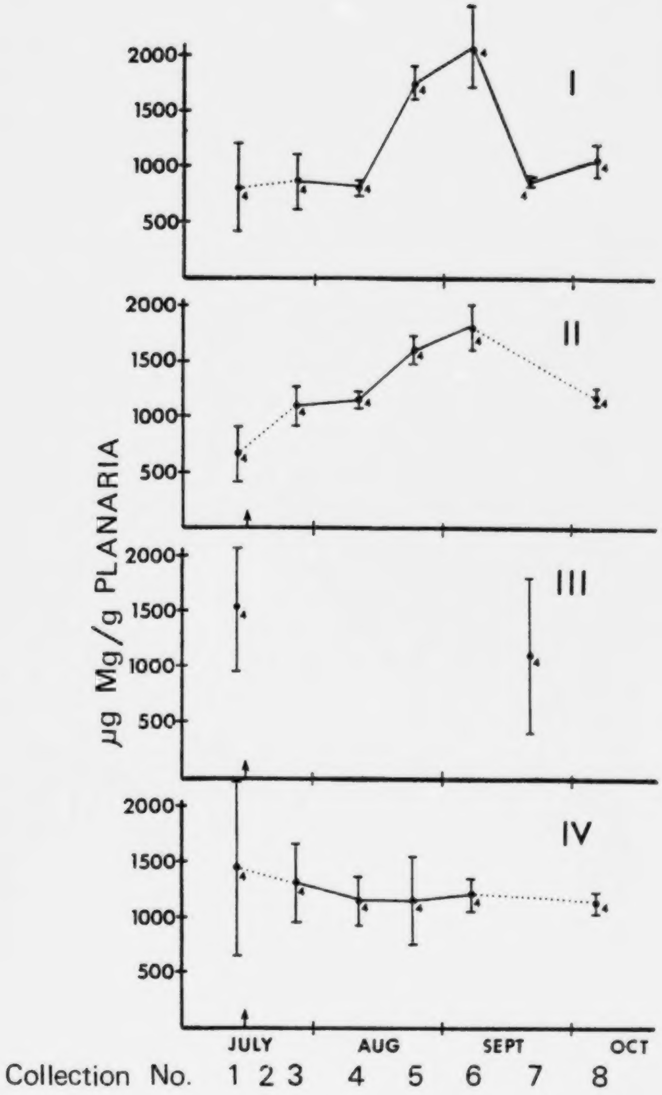

Figure 38. Concentration of magnesium ($\bar{x} \pm$ s.d.) in *Planaria* from ecosystem streams through time. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA. NTA and copper additions to streams begun at arrows. Small numbers indicate sample size.

TABLE 1. Frequency of Determination of Water Chemcial Parameters during Algae Experiments

	No. of measurements per week during indicated Experiment:		
	11/14/74-2/13/75	6/20-10/3/75	3/14-4/15/75
Iron	3	4	daily
Manganese	4	4	daily
Zinc	3	1	4
Copper	1 per month	1	2 per day
Magnesium	1	1	1
Sodium	1	1	-
Potassium	1	1	2
Nitrate	daily	daily	daily
Nitrite	daily	2	daily
Ammonia	3	daily	daily
Phosphate	daily	daily	daily
Silicate	3	daily	daily
Sulfate	1	1	1
Chloride	1	-	daily
Total Alkalinity	1	daily	daily
Calcium	-	1	2

TABLE 2. Relative Retention Times (RRT) and Relative Molar Responses (RMR) of n-Butylesters of twenty-five Carboxylic Acids.

		No. runs	RRT $\bar{x}$	RRT $\bar{x}$	RMR $\bar{x}$	RMR $\bar{x}$	RMR std. error
1.	Malonic	7	0.17	±0.02	0.40	±0.16	±0.05
2.	Succinic	5	0.26	±0.02	0.46	±0.06	±0.03
3.	Fumaric	5	0.27	±0.02	0.41	±0.05	±0.02
4.	Cinnamic	4	0.32	±0.01	0.69	±0.07	±0.03
5.	Malic	5	0.41	±0.01	0.41	±0.03	±0.01
6.	p-Hydroxybenzoic	4	0.44	±0.02	0.43	±0.02	±0.01
7.	MIDA	20	0.50	±0.04	0.76	±0.16	±0.04
8.	IDA	4	0.53	±0.01	0.41	±0.07	±0.03
9.	Tartaric	5	0.55	±0.04	0.30	±0.05	±0.02
10.	Vanillic	5	0.60	±0.04	0.66	±0.13	±0.06
11.	Myristic	10	0.70	±0.09	1.26	±0.50	±0.16
12.	Shikimic	9	0.83	±0.04	0.59	±0.20	±0.07
13.	Palmitic	5	0.94	±0.00	1.13	±0.10	±0.04
14.	Phthalic		1.00		1.00		
15.	Coumaric	5	1.07	±0.00	0.61	±0.06	±0.03
16.	Syringic	4	1.12	±0.00	0.73	±0.10	±0.05
17.	Linoleic	10	1.14	±0.02	1.16	±0.29	±0.09
18.	Linolenic	4	1.16	±0.02	1.29	±0.27	±0.13
19.	Stearic	9	1.17	±0.02	1.50	±0.38	±0.12
20.	Ferulic	10	1.23	±0.03	0.75	±0.16	±0.05
21.	Citric	10	1.29	±0.04	0.93	±0.11	±0.04
22.	Arachidic	4	1.31	±0.06	1.78	±0.07	±0.04
23.	NTA	7	1.36	±0.05	0.81	±0.11	±0.04
24.	Sinapic	10	1.46	±0.09	0.73	±0.22	±0.08
25.	Behenic	10	1.50	±0.08	2.09	±0.46	±0.15

TABLE 3. Evaluation of GC Analytical Procedure Using carboxyl-labeled ^{14}C-NTA.

Sample	DPM/Sample 1	2	3	4	5	Mean	%
Starting solution	187,800	216,850	207,450	198,050	207,450	203,510	100.0
0.1M Formic acid eluate	3,110	4,770	4,200	2,970	3,370	3,634	1.8
16M Formic acid eluate	185,000	185,000	170,000	188,000	147,000	175,000	86.0
Esterified sample	84,400	134,000	166,000	169,300	138,000	139,360	68.5
Sample for GC analysis	119,350	178,950	208,900	170,700	87,200	153,020	75.2

TABLE 4. Chelation of Metals from Sediments by 10^{-3}M NTA (191 ppm).

	White Clay	Red Clay (upstream)	Red Clay (sewage plant)
No. samples	5	6	6
Density of sediment	1.40 ± 0.05	1.41 ± 0.03	1.40 ± .09
ug Fe/g sediment	23400 ± 2920	19800 ± 3400	26700 ± 2600
mg in top cm	777	665	889
ug Cu/g sediment	7.8 ± 1.1	6.1 ± 1.7	14.1 ± 3.8
mg in top cm	.259	.204	.469
ug Zn/g sediment	38 ± 3.7	27.3 ± 2.0	78.3 ± 5.3
mg in top cm	1.26	.917	2.61
ug Mn/g sediment	524 ± 97	810 ± 230	690 ± 107
mg in top cm	17.4	27.2	23.0
Fe - max ug solubilized (NTA)	450	400	2500
% "available"	<0.01	<0.01	<0.01
Cu - mag ug solubilized (NTA)	12.5	11.1	40
% "available"	4.8	5.4	8.5
Zn - max ug solubilized (NTA)	56	67	228
% "available"	4.4	7.3	8.7
Mn - max ug solubilized (NTA)	830	1210	760
% "available"	4.8	4.4	3.3

TABLE 5. NTA Determinations for the Period
November 14, 1974 - February 13, 1975

Desired Concentrations (mg/1)	2.0			20.0		
Microcosm Box #	1	2	5	3	6	7
Zinc-Zincon Method						
$\overline{X}$	1.84	1.67	1.49	19.95	20.23	20.97
N	60	61	61	61	61	61
Maximum	4.9	3.6	3.1	37.6	36.4	30.6
Minimum	0.3	0.2	0.2	0.6	4.2	6.6
# out of 30% range*	24	22	30	12	11	9
SD	0.844	0.720	0.706	6.11	5.50	5.23
Coef. of var.	46.0	43.1	47.3	30.6	27.2	24.9
SE	0.109	0.092	0.090	0.783	0.704	0.669
SE_m (%)	5.92	5.51	6.02	3.92	3.48	3.19
GC Method						
$\overline{X}$	1.54	1.72	1.52	18.7	19.3	19.6
N	85	86	85	85	84	85
Maximum	3.68	4.20	4.50	32.4	29.5	29.5
Minimum	0.06	0.20	0.20	8.1	4.6	6.2
# out of 30% range	43	47	42	18	11	12
SD	0.811	0.822	0.760	4.94	4.53	4.05
Coef. of var.	52.7	47.8	50.0	26.4	23.5	20.7
SE	0.088	0.088	0.082	0.535	0.494	0.439
SE_m (%)	5.71	5.12	5.39	2.86	2.56	2.24

*Previous experiments have shown that in the range of many natural variables this is the degree of variation one may expect under similar conditions.

TABLE 6. NTA Determination for July 4 - September 2, 1975 Experiment

Concentration (mg/l)	Control	2.0	20.0
Microcosm Box #	2 & 8	5 & 7	4 & 6
Zinc-Zincon Method			
$\overline{X}$		1.94	19.4
N		54	57
Maximum		2.5	31.6
Minimum		0.4	2.0
# out of 30% range		3	3
SD		0.39	4.09
Coef. of var.		19.85	20.58
SE		0.053	0.543
SE_m (%)		2.70	2.73
GC Method			
$\overline{X}$	0.06	1.93	18.94
N	27	52	50
Maximum	0.81	3.72	26.3
Minimum	0	0.4	1.4
# out of 30% range		16	8
SD		0.661	4.353
Coef. of var.		34.22	22.98
SE		0.092	0.616
SE_m (%)		4.74	3.25

TABLE 7. Water Chemistries for the Test Chambers (November 14, 1974 - February 13, 1975) Day Length: 10 hr. .03 min. - 9 hr. 21 min. - 10 hr. 38 min.

NTA (mg/l)	Fe (33-35)[1]	Mn (46-49)	Zn (32-35)	Cu (4)	Mg (12)	Ca (12)	Na (13)	K (12)	NO_3N (57-58)	NO_2N (58-59)	NH_3N (36)	PO_4P (58-59)	SiO_2 (33-34)	SO_4 (11)	Cl (10-11)	Tot Alk (14)
#4 Control																
Max.	.46	.850	.014	<.01	8.9	23.7	6.65	2.70	3.44	.021	.060	.053	16.3	19.4	13.2	54.0
Min.	<.01	<.01	<.01	<.01	7.2	16.5	3.90	1.92	2.49	.004	<.01	.006	7.4	15.3	8.3	47.0
Ave.	.170	.186	.003	-	7.90	19.0	5.55	2.25	3.06	.010	.013	.021	13.0	17.7	10.2	50.3
#8 Control																
Max.	.36	.48	.066	.010	8.7	25.0	7.90	2.50	3.52	.028	.160	.062	16.5	18.7	12.6	56.0
Min.	.02	<.01	<.01	<.01	7.0	17.0	4.00	1.95	2.57	.003	<.01	.007	11.4	13.5	8.3	47.0
Ave.	.150	.1384	.007	.002	7.83	19.2	5.75	2.12	3.14	.012	.016	.025	14.2	16.9	10.2	51.1
#1 (2.0)																
Max.	.36	.610	.048	.039	8.9	22.9	7.35	3.06	3.48	.078	.070	.057	16.3	19.0	13.2	57.0
Min.	.04	<.01	<.01	<.01	7.1	17.3	4.26	2.13	2.55	.005	<.01	.009	11.4	14.7	8.3	46.0
Ave.	.19	.14	.013	.014	7.86	19.2	6.18	2.64	2.99	.026	.026	.025	14.2	17.3	10.5	51.2
#2 (2.0)																
Max.	.50	.380	.063	.013	8.7	24.4	7.02	2.78	4.20	.061	.072	.054	16.5	18.8	12.6	54.0
Min.	.04	<.01	<.01	<.01	7.2	17.2	4.13	2.05	2.62	.005	<.01	.006	11.4	13.4	8.3	44.0
Ave.	.19	.18	.014	.003	7.88	19.3	6.09	2.41	3.10	.028	.025	.024	13.9	17.2	10.1	49.7
#5 (2.0)																
Max.	.50	.570	.039	<.01	8.9	23.7	7.47	2.63	3.50	.065	.064	.052	16.0	19.2	12.0	56.0
Min.	<.01	<.01	<.01	<.01	7.1	16.5	4.26	1.95	2.57	.005	<.01	.007	9.4	14.0	8.3	46.0
Ave.	.184	.169	.009	-	7.78	18.7	6.14	2.21	3.09	.033	.022	.021	13.4	17.4	10.3	50.6
#3 (20.0)																
Max.	.46	.444	.050	.025	8.7	25.3	13.74	3.40	3.66	.293	.116	.054	16.3	19.0	12.6	55.0
Min.	.02	<.01	<.01	<.01	7.1	17.0	8.52	2.08	2.57	.004	<.01	.003	11.4	15.1	8.9	47.0
Ave.	.194	.086	.011	.010	7.95	19.9	11.41	2.54	3.13	.075	.058	.022	14.3	17.4	10.6	50.7
#6 (20.0)																
Max.	.61	.59	.043	<.01	8.9	24.4	15.74	2.80	3.48	.078	.148	.046	15.8	19.2	13.8	54.0
Min.	.02	<.01	<.01	<.01	7.1	16.0	8.24	1.98	2.57	.003	<.01	<.002	8.4	15.1	8.3	48.0
Ave.	.222	.143	.011	-	7.85	19.0	11.47	2.35	3.07	.033	.044	.019	13.1	17.6	10.6	51.3
#7 (20.0)																
Max.	.51	.52	.176	<.01	8.5	25.0	13.90	2.79	3.56	.308	.174	.065	15.4	18.5	13.8	56.0
Min.	<.01	<.01	<.01	<.01	7.1	15.5	8.24	2.03	2.62	.005	<.01	.003	10.8	15.1	8.9	46.0
Ave.	.223	.161	.017	-	7.77	18.9	11.47	2.32	3.14	.069	.043	.022	13.7	17.2	10.6	51.4

[1] Number of Determinations

TABLE 8. Water Chemistries for the Experimental Boxes (June 20, 1975 - September 3, 1975) Day Length: 14 hr. 54 min. - 12 hr. 59 min.

	Mn (41)[1]	Fe (41)	Zn (9)	Cu (8)	Mg (8)	Ca (8)	Na (8)	K (8)	PO_4P (42)	SiO_2 (41)	NO_3N (42)	NO_2N (13-17)	NH_3N (41)	SO_4 (8)	Cl (8)	Alk P. (38)	Alk M O (38)
Test Unit #1 - Control Boxes 2 and 8																	
Max.	.19	.35	.500	.02	7.5	21.6	5.81	3.05	.051	16.4	3.07	.014	.018	20.0	14.0	0	56.0
Min.	<.02	<.05	<.01	<.01	6.1	16.0	4.07	1.40	.009	4.2	0.60	.005	<.002	16.2	6.0	0	30.0
Ave.	.067	.14	.069	<.01	7.1	19.3	5.49	2.23	.026	13.7	2.64	.008	.005	17.8	9.3	0	51.1
Test Unit #2 - 2.0 mg/1 Boxes 5 and 7																	
Max.	.22	.34	.418	.014	7.4	20.7	7.70	3.16	.037	16.0	3.22	.075	.075	18.8	13.0	0	58.0
Min.	<.02	.05	<.01	<.01	6.3	15.9	4.84	1.37	.007	4.2	0.55	.006	<.002	17.1	6.0	0	45.0
Ave.	.055	.14	.054	<.01	7.08	19.1	6.34	2.19	.019	12.6	2.76	.030	.011	18.2	10.0	0	51.7
Test Unit #3 - 20.0 mg/1 Boxes 4 and 6																	
Max.	.19	.38	.220	.015	7.4	20.7	14.60	3.30	.043	17.5	3.59	.890	.264	19.4	14.4	0	68.0
Min.	<.02	.05	<.01	<.01	6.4	16.3	8.90	1.77	.007	3.4	0.63	.006	<.002	17.2	8.0	0	46.0
Ave.	.068	.17	.029	<.01	7.13	19.1	11.98	2.49	.017	12.2	2.81	.192	.071	18.2	10.4	0	55.0

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[1] Number of determinations

TABLE 9. Metals in Biomass (mg cation/g Dry Weight) November 1974 - February 1975

	November 14, 1975 (end of seeding)			January 17, 1975 (35 days after seeding)			February 13, 1975 (end of experiment)		
	Control	2 mg/l	20 mg/l	Control	2 mg/l	20 mg/l	Control	2 mg/l	20 mg/l
Mg	4.6	5.1	4.9	4.7	5.2	4.7	3.7	3.7	4.4
Ca	4.6	6.1	5.6	8.7	11.9	8.7	8.3	9.1	11.9
K	3.7	7.2	3.7	6.5	5.9	6.4	3.9	4.5	4.7
*Na	5.6	6.6	3.7	1.0	1.1	1.0	0.65	0.79	.93
*Fe	22.3	30.0	17.9	28.3	39.0	28.3	19.6	22.4	15.7
*Mn	10.6	14.3	1.1	36.9	51.6	36.98	45.9	60.2	74.9
Cu	0.16	0.57	0.21	0.08	0.11	0.09	0.15	0.12	0.088
Zn	0.17	0.3	0.15	0.42	0.26	0.42	0.47	0.17	0.099

*These chemicals were added during the course of all experiments, Fe and Mn to maintain concentrations, Na with the NTA.

TABLE 10. Metals in Algal Biomass - mg cation/gm
July - August 1975

	July 3, 1975 (end of seeding)			August 1, 1975			August 31, 1975		
	Control	2 mg/l	20 mg/l	Control	2 mg/l	20 mg/l	Control	2 mg/l	20 mg/l
Mg	8.49	8.44	9.8	3.9	2.77	3.3	5.60	4.93	4.30
Ca	7.07	7.2	9.66	8.07	5.68	6.23	14.38	12.28	11.72
K	4.59	4.39	5.61	8.04	6.93	7.73	7.19	7.64	7.93
Na	1.33	0.91	1.56	2.16	0.88	1.09	2.32	1.39	1.50
Fe	50.87	52.05	65.1	14.24	10.71	10.21	19.94	15.42	13.51
Mn	3.0	3.1	3.49	29.32	21.23	19.75	48.65	39.75	49.30
Zn	0.31	0.24	0.33	0.45	0.13	0.15	0.61	0.19	0.13
Cu	0.09	0.09	0.13	0.09	0.06	0.06	0.13	0.11	0.11
Ni	0.49	0.54	0.48	0.16	0.09	0.11	0.184	0.136	0.236

TABLE 11. Slide Observations, November 14 - December 10, 1974
End of Seeding

Box #	Approximate NTA (mg/l)	Observations (November 14, 1974)
4	Control	As in #1, except diversity less *Synedra ulna* and *Nitzschia linearis* - dominants
8	Control	As in #1, but much less *Synedra*
1	2.0	Diatoms - dominant, diversity - good (*Cyclotella* sp., *Achnanthes lanceolata*, *Synedra ulna*, *Nitzchia linearis*, *N. acicularis*) Greens - frequent *Stigeoclonium protonema* - common Blue-greens - rare
2	2.0	As in #1
5	2.0	As in #1, except *Chlamydomonas*, *Cyclotella* and *Stigeoclonium* - rare
3	20.0 (19.8)	As in #1
6	20.0 (19.8)	As in #5
7	20.0 (19.8)	As in #1

(Continued)

TABLE 11. (Continued)
(4 weeks after Seeding)

Box #	Approximate NTA (mg/l)	Observations, (December 10, 1974)
3	20.0 (19.3)	Diatoms - dominant, diversity less than #4 and #8 *Achnanthes lanceolata*, *Synedra ulna*, *Nitzschia linearis* and *Melosira varians* - dominant. Some indication of pavement-like growth for *Achnanthes* along with many empty frustules of this species. Overall growth somewhat less than #4 and #8 Greens: *Stigeoclonium* protonema common *Chlamydomonas* frequent Blue-greens: frequent to common
7	20.0 (19.8)	As in #3, fewer empty frustules of *Achnanthes*
6	20.0 (20.3)	As in #3, but diatom diversity better than #3 and #7

(Continued)

TABLE 11. (Continued)
(4 weeks after Seeding)

Box #	Approximate NTA (mg/l)	Observations, (December 10, 1974)
4	Control	Diatoms - dominant, diversity good. Melosira varians, Achnanthes lanceolata, Nitzschia linearis, Cymbella, Synedra ulna all common to abundant. Cyclotella, Diatoma and Cocconeis frequent at best. Greens: Stigeoclonium protonema common Chlamydomonas sp. nonmotile palmeloid colonies - common, other greens - infrequent Blue-greens: Microcoleus - frequent at best Schizothrix - frequent Oscillatoria - infrequent Protozoa: Vorticella, Difflugia, ciliates and rotifers - common
8	Control	As in #4, but Schizothrix frequent - common; Scenedesmus infrequent
1	2.0 (1.7)	Diatoms - dominant, diversity good, similar to #4 and #8 Greens: Scenedesmus - common otherwise similar to #4 and #8
5	2.0 (1.7)	As in #1, but growth less than any other NTA level box Blue-greens: Microcoleus - common
2	2.0 (1.5)	As in #1

(Continued)

TABLE 11. (Continued)
(6 weeks after Seeding)

Box #	Approximate NTA (mg/l)	Observations, (December 26, 1974)
4	Control	Diatoms - dominant, growth good, diversity good, Melosira varians, Achnanthes lanceolata - abundant Synedra ulna, Cymbella, Nitzschia linearis, various species of Navicula, Cocconeis placentula - all common. Greens: rare, except Stigeoclonium protonema common Blue-greens: Schizothrix calcicola common, Microcoleus infrequent
8	Control	As in #4
1	2.0 (1.7)	Diatom community as in controls, Blue-greens: similar to controls, Greens: Scenedesmus - frequent to common Otherwise as in #4 and #8
2	2.0 (1.7)	As in #1
5	2.0 (1.5)	As in #1
3	20.0 (19.3)	Diatoms - dominant, growth good, diversity not as good as in controls, pavement-like growth habit observed. Greens and Blue-greens: similar to control and 2.0 mg/l boxes.
6	20.0 (19.8)	As in #3
7	20.0 (20.3)	As in #3

TABLE 12. Biomass, Chlorophyll and ^{14}C Determination (November 14, 1974 to February 13, 1975)

	Approximate NTA levels (mg/1)										
	0	0	2.0	2.0	2.0	20.0	20.0	20.0	$\bar{x}$	Coef. var.	S.E. Mean (%)
End of Seeding (Nov. 12)											
Ash Free dry weight (mg)	29.6	9.1	11.6	10.7	22.9	4.0	58.3	16.8	20.38	85.2	30.1
Percent of volatile material	10.3	11.7	9.4	13.0	10.1	13.0	8.2	11.1	10.85	15.7	5.5
Six weeks after Seeding (Dec. 23)											
Chlorophyll a/c	7.4	6.5	8.8	8.2	6.7	7.1	7.3	7.4			
35 days after Seeding (Jan. 17)											
Ash free dry weight (mg)	64.9	76.4	58.5	62.3	59.9	56.6	52.6	52.5			
Percent of volatile material	27	26	29	28	28	34	27	36			
End of Experiment (Feb. 11-13)											
Ash Free dry weight (mg)	53.6	41.2	34.9	40.4	43.1	44.2	29.1	75.9			
Percent of volatile material	27	25	34	31	29	37	30	33			
Chlorophyll a/c	7.5	6.8	6.5	6.8	6.9	7.4	6.6	5.8			
DPM/ug Chlorophyll a	1094	1270	1120	1449	1451	608	1626	940			

TABLE 13. Detailed Analysis of Diatom Populations by the Truncated Normal Curve Technique[1] (November 12, 1974 - February 13, 1975)

Approximate NTA Concentration (mg/l)	Control		Control		2.0		2.0		2.0		20.0		20.0		20.0	
Microcosm Box #	4		8		1		2		5		3		6		7	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
Position of modal interval	2-3	3	2-3	3	3	3	3	2-3	2-3	3	3	3	3	3	3	2-3
# of intervals	10	10	10	11	10	11	10	11	9	10	10	11	10	10	10	10
# of observed species	129	75	133	78	114	75	112	79	122	80	124	71	118	75	132	75
# of species in modal interval	20.9	11.1	21.1	10.8	17.9	11.1	18.2	11.0	20.1	12.0	19.9	9.8	19.0	11.7	21.0	11.7
σ^2 (sigma squared)	8.3	10.7	7.5	11.1	7.8	12.2	7.6	13.0	8.2	9.8	8.0	11.0	8.4	8.8	8.2	9.5
Position of the mode	2.0	2.4	2.0	2.8	2.3	2.2	2.4	2.1	2.0	2.3	2.1	2.7	2.2	2.4	2.1	2.0
The theoretical universe	153.9	91.0	155	90.2	125.2	97.3	125.3	100.2	146.9	94.0	142.3	81.4	139.3	87.0	151.9	91.2
Total # specimens counted	4206	4098	4000	4787	4583	4034	3942	4502	3519	3156	4112	3417	3810	3572	4298	3048
# of species $\geq$ 10%	3	2	3	3	3	4	3	2	2	2	3	4	2	3	3	3

B - end of seeding - November 12, 1974

A - end of experiment - February 13, 1975

[1] Patrick, R., M. Hohn and J. Wallace, 1954.

TABLE 14. Distribution of the more Common Diatom Species ($\geq$ 1.67% of Specimens Counted)

Approximate NTA Concentration(mg/l)	Control		Control		2.0		2.0		2.0		20.0		20.0		20.0	
Microcosm Box #	8		4		1		2		5		3		6		7	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
Achnanthes lanceolata	12.5	22.6	12.1	17.6	20.3	23.7	15.0	20.7	10.6	8.8	7.0	30.7	11.8	25.4	8.2	21.7
Nitzschia linearis	18.8	-	16.2	-	16.5	-	17.4	-	14.1	2.7	18.7		14.2	-	15.5	-
Nitzschia frustulum v. perminuta	-	5.4	2.6	8.3	-	3.5		7.4		6.6		6.6	-	-	-	3.6
Nitzschia kutzingiana	-	3.9	-	4.7	-	3.2		3.3		10.5		11.8	3.2	12.9	-	5.2
Achnanthes minutissima	-	12.7	2.8	7.2	-	11.9	-	7.6	2.5	5.9		9.6	3.8	9.8	-	8.1
Navicula genovefea	10.4	-	9.9		9.1	-	11.3	-	7.5	-	10.8		7.5	-	13.1	-
Navicula secreta v. apiculata	9.4	6.2	7.5	3.1	9.1	4.3	8.3	6.3	8.3	8.6	7.8	6.0	9.9	4.4	8.1	8.3
Cyclotella meneghiniana	8.7	-	10.3		7.1	-	7.8		8.4	-	11.9		5.8	-	10.4	-
Synedra ulna	6.2	8.5	5.4	3.0	6.4	-	3.1	-	5.2	2.9	5.8		5.7	3.9	6.2	-
Melosira varians	-	-	2.4	5.1	1.6	11.2	2.3	9.9	2.5	7.9		10.0	-	4.6	2.8	12.7
Nitzschia fonticola	-	13.4		20.4	-	13.9		16.1		17.3		11.2		11.0	-	12.5
Amphora ovalis v. pediculus	-	2.3		2.0	-	6.0		4.1				6.8		5.2	-	3.1
Suriella angustata	2.1	-									2.3					
Nitzschia dissapata				4.2												
Navicula minima		3.5										3.5				
Navicula tripunctata																4.2
Navicula seminulum												3.7				

B - before treatment-November 12, 1974 A - after treament-February 12, 1975

- = <1%

blank space = not seen

TABLE 15. Microscopic Examination of Communities

	July 2	July 31
Control:		
Boxes 8 and 2	Achnanthes lanceolata - very common Melosira varians - very common Cocconeis placentula - common Gomphonema sp. - frequent Cymbella cuspidata - frequent + Nitzschia linearis - common Stigeoclonium lubricum protonema Schizothrix calcicola - frequent	Melosira varians - abundant Achnanthes lanceolata - abundant Cocconeis placentula - abundant Cymbella cuspidata - very common Spirogyra sp. - very common Microcoleus vaginatus - frequent - Schizothrix calcicola - frequent -
1.94 mg/l NTA		
Boxes 5 and 7	Achnanthes lanceolata - very common Melosira varians - very common Gomphonema parvulum - common Cymbella cuspidata - common Nitzschia linearis - very common Stigeoclonium lubricum - rare Schizothrix calcicola - rare	Melosira varians - abundant Achnanthes lanceolata - very common Achnanthes minutissima - very common Cocconeis placentula - very common pale) Nitzschia linearis - common Cymbella cuspidata - common Gomphonema parvulum - frequent - Spirogyra sp. - frequent - Schizothrix calcicola - rare Microcoleus vaginatus - rare
19.2 mg/l NTA		
Boxes 4 and 6	Achnanthes lanceolata - very common Melosira varians - common Cocconeis placentula - common Nitzschia linearis - common Diatoma vulgare - frequent + Cymbella cuspidata - common Navicula viridula - frequent + Stigeoclonium lubricum - frequent Sphaerocystis - frequent + Schizothrix calcicola - rare	Melosira varians - abundant Achnanthes minutissima - common + Cymbella cuspidata - frequent Cocconeis placentula - poor condition Nitzschia linearis - common - Gomphonema sp.-frequent - Schizothrix calcicola - common Microcoleus vaginatus - frequent

TABLE 16. Analysis of Diatom Population Using Truncated Normal Curve Factors

Approximate NTA Concentration(mg/l)	Control		Control		2.0		2.0		20.0		20.0	
Microcosm Box #	2		8		5		7		4		6	
	B	A	B	A	B	A	B	A	B	A	B	A
Position of mode	3	2-3	3	3	2-3	3	2-3	3	3	3	3	2-3
# of intervals	11	12	10	13	10	12	11	13	10	12	10	11
# of observed species	120	67	120	68	118	76	113	71	116	55	107	56
# of species in modal interval	18.2	8.6	18.1	8.6	17.9	10.2	17.1	9.1	17.3	7.5	16.4	7.5
σ^2 (sigma squared)	10.6	17.8	10.7	16.9	10.4	15.5	11.8	16.6	9.4	12.4	9.0	16.1
Position of the mode	2.2	2.0	2.1	2.3	2.1	2.3	2.0	2.6	2.5	2.7	2.6	2.0
The theoretical universe	148.5	91.7	150.0	88.5	146.3	100.3	148.6	93.1	132.6	66.0	123.0	76.4
Total # specimens counted	5486	9261	5783	15091	5283	13102	6322	14959	5311	6309	5052	6784
# of species $\geq$ 10%	2	3	2	2	1	4	2	4	2	3	2	3

B = Before experiment (July 3, 1975)
A = After experiment (September 3, 1975)

TABLE 17. Distribution of the more Common Diatom Species

Approximate NTA Concentration (mg/l)	Control				2.0 mg/l				20.0 mg/l			
Microcosm Box #	2		8		5		7		4		6	
	B*	A	B	A	B	A	B	A	B	A	B	A
Achnanthes lanceolata	19.9	4	15.6	1	17.5	2	23.3	2	14.2	4	15.8	5
Navicula secreta v. apiculata	11.1		11.8		9.4		11.3		10.3		9.5	
Navicula genovefea	5.4		7.2		4.3		7.0		4.1		6.3	
Nitzschia frustulum v. perminuta	3.5		3.8		2.6		5.1		4.5		3.2	
Navicula rhynchocephala v. germainii	3.4		4.0		3.5		4.4		3.8		4.6	
Navicula pelliculosa	2.1		3.3		3.7		3.8		3.8		1.0	
Navicula minima	3.5	20	3.7	5	3.5	14	3.5	11	4.5	11	1.6	12
Melosira varians	8.1	4	6.4	1	7.1	20	2.2	13	8.0	1	15.5	22
Rhoicosphenia curvata	3.2		3.4		4.3		2.8		4.1		3.5	
Nitzschia kutzingiana	2.6		2.0		4.9		3.1		1.7		3.4	
Navicula gregaria	1.5		1.3		1.7		1.0		3.8		1.4	
Achnanthes minutissima		27		46		28		28		49		28
Nitzschia fonticola		17		18		12		16		10		9
Achnanthes subhudsonis v. krae.		5		1		1		2		1		2
Cocconeis placentula v. euglypta		4		3		3		3		3		3
Nitzschia tropica		4		6		4		5		3		3
Gomphonema MN$_3$		2		1		2		3		4		3
Others	42.1	27	51.1	16	41.9	15	41.5	19	43.2	15	41.6	11

*B - Before experiment, ≥ 3.2% of specimens counted A - After experiment, ≥ 1% of specimens counted

TABLE 18. Effects of NTA on Algal Communities

Approximate NTA Concentrations mg/l	Control		2.0		20.0				
Microcosm Box #	2	8	5	7	4	6	$\overline{x}$	coef. var.	SE_m (%)
July 3 (end of seeding)									
Ash-free dry weight (mg)	10.9	11.0	15.4	10.6	8.5	10.0	11.1	20.9	8.5
% volatile material	21.1	17.7	15.2	16.6	18.7	22.1	18.6	14.2	5.8
August 1 (30 days after seeding)									
Ash-free dry weight (mg)	22.9	21.0	19.8	19.4	22.4	16.7			
% volatile material	38.4	38.3	35.2	32.1	36.5	37.6			
Chlorophyll a/c	18.2	17.9	19.7	15.0	14.0	12.7			
September 3 (60 days after seeding)									
Phycocyanin (mg/side of slide)	80.0	274.0	60.9	125.0	33.3	36.1			
Chlorophyll a/c	15.3	17.6	12.4	4.4	4.4	4.6			
% volatile material	43	44	43	43	45	49			
Ash-free dry weight (mg)	38.4	59.9	24.9	43.7	26.7	14.0			
DPM/ug chlorophyll a	1019	2388	1242	1846	1196	1832			

TABLE 19. Water Chemistries for the Experimental Boxes (March 14 - April 15, 1975) Data in mg/l Day Length: 11 hr. 52 min. to 13 hr. 15 min.

	Mn	Fe	Zn	Mg	Ca	Na	Cu	K	PO_4P	SiO_2	NO_3N	NO_2N	NH_3N	SO_4	Cl	Alk-P	Tot Alk
	(21-22)*	(21-22)	(15-17)	(4-5)	(9-10)	(4-5)	(8-45)**	(9)	(22-23)	(21-23)	(22-23)	(22-23)	(20)	(4-5)	(19-20)	(21-22)	(21-22)
#4 Control																	
Max.	.30	.17	.024	7.7	18.0	5.30	no	2.49	.022	14.5	3.57	.034	.032	19.5	11.0	0	49.0
Min.	<.01	.04	<.01	6.2	10.5	4.47	deter.	1.65	.004	7.9	1.83	.007	<.005	16.4	8.0	0	35.0
Ave.	.056	.086	.007	7.24	15.6	5.04		1.99	.011	12.4	3.02	.013	<.005	18.2	9.6	0	44.5
#7 NTA																	
Max.	.32	.18	.027	7.4	17.8	5.81	<.01	2.25	.019	14.0	3.37	.050	.042	19.4	12.0	0	49.0
Min.	<.01	.13	<.01	6.8	12.7	4.95	<.01	1.67	.022	9.0	2.35	.011	<.005	17.1	9.0	0	38.0
Ave.	.048	.093	.009	7.2	15.7	5.49	<.01	1.91	.011	12.3	3.03	.020	.008	18.4	9.8	0	44.9
#10 NTA																	
Max.	.44	.21	.112	7.3	19.2	5.63	<.01	2.15	.028	13.9	3.34	.021	.044	19.7	12.0	0	50.0
Min.	<.01	.02	<.01	6.7	13.0	5.30	<.01	1.70	.005	9.4	2.44	.007	<.005	17.9	8.0	0	37.0
Ave.	.095	.110	.026	7.03	15.6	5.51	<.01	1.87	.014	12.7	3.05	.014	.006	18.9	10.3	0	44.7
#2 NTA-Cu																	
Max.	.48	.25	.016	7.5	17.8	5.80	.150	2.90	.020	13.9	3.43	.030	.023	20.6	12.0	0	48.0
Min.	<.01	.05	<.01	7.0	14.4	5.23	.058	1.67	.004	9.0	2.30	.007	<.005	16.0	8.0	0	37.0
Ave.	.129	.131	.006	7.30	15.9	5.59	.102	2.20	.012	12.4	2.76	.015	<.005	18.7	10.1	0	44.4
#6 NTA-Cu																	
Max.	.35	.22	.016	7.5	17.8	5.80	.132	2.34	.021	13.8	3.46	.037	.027	19.6	12.0	0	49.0
Min.	<.01	.03	<.01	6.7	11.6	5.22	.070	1.64	.005	8.8	2.28	.009	<.005	17.9	8.0	0	38.0
Ave.	.109	.116	.007	7.2	15.4	5.59	.098	1.93	.011	12.4	3.04	.017	<.005	18.9	10.3	0	45.0
#8 Cu																	
Max.	.50	.25	.016	7.4	18.0	5.35	.134	2.05	.028	14.2	3.46	.020	.050	20.2	11.0	0	48.0
Min.	.03	.04	<.01	6.8	10.4	4.79	.020	1.72	.011	9.5	2.49	.006	<.005	15.9	9.0	0	41.0
Ave.	.186	.122	.006	7.2	15.4	5.15	.075	1.86	.018	13.0	3.08	.014	.008	18.5	10.2	0	44.7
#9 Cu																	
Max.	.48	.24	.54	7.2	18.0	5.30	.122	2.09	.022	14.0	3.44	.027	.025	20.2	12.0	0	51.0
Min.	.01	.06	<.01	6.9	11.8	4.66	.037	1.64	.007	9.2	2.28	.005	.005	17.4	8.0	0	37.0
Ave.	.155	.115	.020	7.10	15.3	5.10	.073	1.87	.016	12.9	3.07	.013	.005	19.1	10.1	0	45.2

* Number of Determinations

** 45 determination where Cu was added, otherwise 8-10.

TABLE 20. Determinations for NTA and Cu during the Period March 12 - April 15, 1975

	Control	NTA-Cu		NTA		CU	
Microcosm Box #	4	2	6	7	10	8	9
NTA, GC technique							
$\overline{X}$ (mg/l)	0.02	1.76	1.60	1.25	1.47	0.015	0.006
N	7	27	27	27	26	7	7
SD	0.03	0.49	0.60	0.45	0.50	0.02	0.007
Coef. of var.	157	27.6	37.3	35.9	34.2	163	129
SE	0.01	0.09	0.11	0.09	0.10	0	0.003
SE_m (%)	49.8	5.3	7.2	6.9	6.7	0	46.3
Max.	0.09	2.71	2.70	2.12	2.96	0.07	0.016
Min.	0	0.82	0.52	0.46	0.86	0	0
# out of 30% range		8	10	9	8		
NTA, Zinc-Zincon technique							
$\overline{X}$ (mg/l)		1.53	1.41	1.31	1.45		
N		23	23	23	23		
SD		0.46	0.44	0.48	0.59		
Coef. of var.		30.5	31.1	36.8	40.7		
SE		0.10	0.09	0.10	0.12		
SE_m (%)		6.4	6.5	7.6	8.5		
Max.		2.1	2.1	2.2	3.1		
Min.		0.4	0.4	0.7	0.6		
# out of 30% range		4	4	6	8		
Cu							
$\overline{X}$ (ug/l)		102	98			75	73
N		41	41			41	41
SD		24	14.7			25.8	19.7
Coef. of var.		23.1	15.1			34.1	26.8
SE		4	2.2			4	3
SE_m (%)		3.5	2.3			5.3	4.1
Max.		150	132			134	122
Min.		58	70			20	37
# out of 30% range		3	0			8	6
Molar ratio of Cu-NTA							
NTA by GC determination		5.8	5.5				

TABLE 21. Microscopic Examination of Communities

March 15	March 22
Control:	Control:
Navicula viridula v. avenacea - dominant	Navicula viridula v. avenacea - dominant
Achnanthes lanceolata - common	Nitzchia palea - common
Nitzschia palea - common	Achnanthes lanceolata - frequent +
Diatoma vulgare - frequent	Melosira varians - frequent +
Ulothrix zonata - frequent	Synedra ulna - frequent
Stigeoclonium lubricum protonema - frequent	Stigeoclonium lubricum protonema - frequent +
Unicellular green - rare	Ulothrix zonata - frequent +
Microcoleus vaginatus - rare	Microcoleus vaginatus - rare
NTA:	NTA:
Navicula viridula v. avenacea - dominant	Navicula viridula v. avenacea - dominant
Nitzschia palea - common	Nitzschia palea - very common
Achnanthes lanceolata - common	Achnanthes lanceolata - common
Synedra ulna - frequent	Synedra ulna - frequent
Stigeoclonium lubricum protonema - frequent	Melosira varians - frequent +
Unicellular green - frequent	Nitzschia linearis - frequent
Microcoleus vaginatus - rare	Stigeoclonium lubricum protonema - common
Schizothrix calcicola - rare	Ulothrix zonata - frequent
	Microcoleus vaginatus - rare
NTA-Cu:	NTA-Cu:
Navicula viridula v. avenacea - dominant	Navicula viridula v. avenacea - dominant
Nitzschia palea - common	Nitzschia palea - common
Achnanthes lanceolata - common	Achnanthes lanceolata - frequent
Diatoma vulgare - frequent	Meridion circulare - common
Ulothrix zonata - frequent	Stigeoclonium lubricum protonema - frequent
Unicellular green - locally common	Oedogonium sp. - rare
Stigeoclonium lubricum protonema - frequent	
Cu:	Cu:
Navicula viridula v. avenacea - dominant	Diatoms in very poor condition
Nitzschia palea - v. common	Stigeoclonium lubricum protonema - frequent + in good condition
Achnanthes lanceolata - common	Schizothrix calcicola - frequent
Stigeoclonium lubricum protonema - frequent	Microcolaus vaginatus - rare to frequent
Unicellular green - frequent	
Microcoleus vaginatus - rare	

TABLE 22. Detailed Analysis of Diatom Populations by the Truncated Normal Curve Technique

Tests	Control		Cu-NTA				NTA			
Microcosm Box #	4		2		6		7		10[1]	
	B	A	B	A	B	A	B	A	B	A
Position of modal interval	3	3	3	3	2-3	3	2-3	2-3	3	4
# of intervals	11	11	11	11	12	11	11	12	12	11
# of observed spp.	86	77	70	72	88	76	72	81	86	62
# of spp. in model interval	10.8	10.8	10.0	10.1	11.6	10.2	10.3	11.6	12.1	8.8
σ^2 (sigma squared)	12.4	12.4	13.0	11.8	15.9	14.8	13.2	12.4	13.1	8.1
Position of the mode	2.2	2.2	2.3	2.5	2.0	2.2	2.1	2.1	2.3	3.8
The theoretical universe	95.1	95.1	90.3	86.8	117	98.1	94.4	103	109.8	63.5
Total # specimens counted	6677	5640	5290	5716	10070	7418	4989	7124	8378	5610
# spp > 10%	4	2	4		3	3	4	3	4	2

* B - before experiment
A - end of experiment

[1] Zinc was very high (2.9 x box 7) & probably caused species reduction.

TABLE 23. Distribution of the more Common Species
(≥ 1.7% of the Specimens Counted)

Microcosm Box #	4		2		6		7		10	
Concentration	Control		Cu-NTA				NTA			
	B	A	B	A	B	A	B	A	B	A
Nitzschia diserta	26.9	26.7	20.8	25.8	13.1	22.2	13.2	33.4	27.1	27.3
Navicula secreta v. apiculata	11.4	21.1	16.4	20.6	29.0	17.6	22.1	21.6	12.2	25.5
Navicula viridula v. avenacea	11.1	8.6	11.7	8.9	8.9	14.5	12.6	13.9	12.6	1.7
Achnanthes lanceolata	5.1	6.3	2.8	5.9	3.4	3.8		3.2	3.8	8.8
Nitzschia kutzingiana	11.9	4.0	14.0	3.6	14.1	5.1	13.1	1.0	11.5	3.0
Achnanthes minutissima		2.5		2.3		2.9		4.6		4.4
Synedra rumpens v. meneghiniana		3.5	2.2	1.0	2.1	-	3.7	1.6		-
Synedra rumpens v. familiaris		1.7		1.6		3.2		-		-
Navicula pelliculosa		1.6		2.2	1.9	2.6	2.8	-		4.2
Nitzschia frustulum v. perminuta		3.4		1.2		2.3		1.4		3.1
Navicula paucivisitata		-		1.4		3.4		-		1.1
Meridion circulare		1.5	2.6	7.1	2.5	3.7		1.7		-
Fragilaria vaucheriae		1.1		3.4		3.6	2.2	1.3	1.7	2.3
Melosira varians		5.4		-		1.6		2.5		-
Nitzschia communis v. hyalina		-		2.8		1.4		-		2.7
Nitzschia linearis	3.3								4.3	
Suriella minuta			3.6				2.8			
Suriella ovata									2.0	
Navicula minima	4.2		2.9						4.3	
Navicula genovefea	3.3				2.6		2.2		4.2	
Others	22.9	18.5	23.1	19.6	22.5	19.9	25.3	17.5	16.7	18.6

(-) Denotes less than one percent, but present
B - before experiment - March 12, 1975
A - after experiment - April 15, 1975

TABLE 24. Biomass, Chlorophyll and ^{14}C Determination

Test	Control	NTA		Cu-NTA		Cu	
Microcosm Box #	4	7	10	2	6	8	9
March 13, 1975 (end of seeding)							
Chlorophyll a/c	9.3	8.6	9.9	10.1	10.9	9.6	9.4
Percent volatile material	17.2[1]	15.9	12.9	16.4	16.8	19.3	20.0
Ash free dry weight (mg)	64.6	100.6	80.2	88.0	47.6	48.1	53.3
March 22, 1975 (9 days after seeding)							
Percent volatile material	21.4	26.2	19.0	36.8	19.4	14.8	16.0
Ash free dry weight (mg)	39.6	46.8	33.5	30.6	39.0	44.3	59.5
April 14-16, 1975 (end of experiment)							
Chlorophyll a/c	9.1	8.1	7.0	9.1	9.2	[2]	[2]
Chlorophyll a (mg/side of slide)	0.83	0.82	0.71	0.75	0.81	0.01	0.07
Chlorophyll c	0.09	0.10	0.10	0.08	0.09	0.01	0
Ash free dry weight (mg)	79.7	84.5	57.4	60.3	68.5	22.1	4.4
Percent volatile material	30.3	29.5	31.8	35.4	33.8	14.0	22.0
Phycocyanin (ug/side of slide)	7.8	7.5	8.0	7.6	8.0	8.0	8.3
DPM/ug chlorophyll a	1613	1366	1605	1344	2446	44	0

[1] Considerable silt resulting in low volatile to non-volatile ratio.
[2] Due to poor condition of diatoms ratio not meaningful

TABLE 25. Metals in Algal Biomass (mg cation/g)
March - April 1975

	End of Seeding						One week in experiment				End of Experiment			
	Control	NTA	Cu-NTA	Cu	coef of var.	SE_m	Control	NTA	Cu-NTA	Cu	Control	NTA	Cu-NTA	Cu
Mg	5.84	5.95	4.28	6.93	20.1	7.6	6.50	5.21	6.61	5.57	5.18	5.11	5.43	6.56
Ca	4.53	3.86	3.54	4.68	12.4	4.7	4.92	4.57	5.31	4.16	3.42	4.09	4.18	3.71
Fe	31.58	29.31	22.71	34.32	20.0	7.2	44.44	25.62	38.79	33.63	20.08	17.72	18.98	35.54
K	3.92	3.93	2.98	5.32	24.3	9.2	4.48	3.98	4.7	3.49	8.58	6.62	8.93	3.91
Mn	1.86	1.49	1.18	1.48	20.3	7.7	4.80	5.35	3.59	3.18	9.29	8.34	11.06	6.37
Na	0.57	0.57	0.47	0.74	20.9	7.9	0.83	0.62	0.73	0.58	0.87	0.87	0.91	1.07
Zn	0.22	0.18	0.11	0.22	31.6	11.9	0.29	0.13	0.12	0.37	0.39	0.13	0.12	0.29
Cu	0.12	0.10	0.09	0.14	18.7	7.1	0.20	0.11	0.22	1.85	0.10	0.08	0.43	5.95

TABLE 26. NTA Concentration in Microcosms
March 12 - April 24, 1975

Desired NTA concentration (mg/l)	Achieved concentration (mg/l) Mean ± Standard Deviation	Achieved concentration (mg/l) Range	Number determinations	Number Determinations ±30 % mean
Gas Chromatographic determination				
0.02	0.09 ± 0.14	0 .63	41	6
0.2	0.16 ± 0.14	0.07 - .76	34	13
2.0	1.20 ± 0.50	0.23 - 2.10	43	23
20	18.70 ± 8.70	0.10 - 41.90	42	21
0 (Control)	0.01 ± 0.02	0 0.09	13	
Zinc-Zincon determination				
2.0	1.4 ± 0.5	0.9 - 2.2	30	
20	18.2 ± 7.5	0.5 - 32.8	33	

TABLE 27. NTA degradation by bacterial populations exposed to different NTA concentrations for four weeks.

Desired NTA Concentration for Assay	Degradation Rate (ng/hr/cm^2)					
	EXPERIMENTAL FLASK:					
	A	B	C	D	E	Dead Cell Control
0	0.02	0.02	0.02	0.02	0.02	0.02
.02	0.26	0.27	0.16	0.21	0.27	0.02
.2	6.5	2.7	3.5	4.3	4.8	0.03
2	13.6	20.1	12.7	10.4	6.9	0.14
20	18.4	15.3	20.6	10.3	14.4	1.9

TABLE 28. NTA Concentration in Microcosms
July 2 - August 14, 1975

Desired NTA concentration (mg/l)	Achieved Concentration (mg/l)		Number Determinations	Number Determination ± 30 % mean
	Mean ± Standard Deviation	Range		
Gas Chromatographic				
0.02	0.06 ± 0.09	(0 - 0.38)	39	3
0.2	0.17 ± 0.16	(0.01 - 0.75)	37	10
2.0	1.70 ± 0.68	(0.62 - 3.30)	30	15
20	20.10 ± 6.20	(14.10 -26.10)	29	22
0 (Control)	0.03 ± .09	(0 - 0.39)	25	3
Zinc-Zincon Determination				
2.0	1.9 ± 0.5	(1.1 - 3.1)	43	32
20	20.3 ± 5.4	(7.0 -39.2)	38	32

TABLE 29. Effect of cadmium, zinc, lead, and nickel ions and NTA-chelates of the same on the metabolism of ^{14}C glucose by heterotrophic bacteria from White Clay Creek. Metal ions present at 1 mg/l, NTA at 4 mg/l.

Date of Experiment	Assay Temperature (^oC)	Inoculum (10^4 cells/ ml)	^{14}C Incorporation as Percent: metal chelate				Percent Inhibition	^{14}C Incorporation as Percent: Chelate/control
			metal chelate	metal control	metal NTA	Mean		
Cadmium								
3/4	9	3.18	40	46	42	43	57	115
3/5	9	3.18	39	39	32	37	63	100
4/11	18	4.08	35	54	35	41	59	154
4/15	18	1930.00	69	61	67	66	34	89
4/18	18	40.80	69	59	56	61	39	85
10/2	15	31000.00	14	11	23	16	84	79
Zinc								
3/4	9	3.18	29	33	30	31	69	114
3/5	9	3.18	34	35	29	33	67	103
4/11	18	4.08	47	75	49	57	43	159
4/15	18	1930.00	59	71	77	69	31	119
4/18	18	40.80	63	66	63	64	36	105
9/30	15	260.00	36	34	39	36	64	94
10/1	15	200.00	59	54	59	57	43	91
Lead								
9/10	20	12.00	82		81	82	18	98
9/12	20	23.00	30	59	40	43	57	194
9/16	20	400.00	76		86	81	19	97
10/7	15	0.68	50	40	41	44	56	79
10/28	20	38.00	12	13	14	13	87	105
Nickel								
9/10	20	12.00		84	33	47	53	49
9/12	20	53.00	29	58	40	42	58	200
9/16	20	400.00	32	47	33	38	62	147
9/16	20	92.00	25	24	24	24	76	96

TABLE 30. Effect of copper ions and copper-NTA chelates on the Metabolism of ^{14}C glucose by heterotrophic bacteria from White Clay Creek. Metal ions present at 1 mg/l, NTA at 4 mg/l.

Date of Experiment	Assay Temperature (°C)	Inoculum (10^4 cells/ml)	^{14}C Incorporation as Percent: metal/chelate	metal/control	metal/NTA	Mean	Percent Inhibition	^{14}C Incorporation as Percent: chelate/control
3/4	9	3.18	13	12	11	12	88	91
3/5	9	3.18	21	18	15	18	82	85
4/11	18	4.08	<1	<1	<1	<1	99	111
4/15	18	1930.00	4	4	4	4	96	93
4/18	18	40.80	4	3	3	3	97	77
9/10	20	12.00	2	4	<1	2	98	182
9/12	20	23.00	4	4	3	4	96	100
9/16	20	400.00	42	19	14	25	75	46
9/29	15	960.00	3	3	3	3	97	93
10/17	15	23.00	1	1	1	1	99	100
10/21	20	52.00	2	1	2	2	98	62
10/24	20	92.00	3	3	2	3	97	100

TABLE 31. Effect of 4 mg/l NTA (probably present as Ca or Fe chelates) on metabolism of ^{14}C glucose by heterotrophic bacteria from White Clay Creek.

Date of Experiment	Assay Temperature (°C)	Inoculum (10^4 cells/ml)	^{14}C Incorporation as Percent: NTA/control
3/4	9	3.18	111
3/5	9	3.18	122
4/11	18	4.08	153
4/15	18	1930.00	92
4/18	18	48.00	105
9/10	20	12.00	255
9/12	20	23.00	157
9/16	20	400.00	136
9/16	20	92.00	100
9/29	15	960.00	100
9/30	15	260.00	87
10/1	15	200.00	92
10/2	15	31000.00	48
10/7	15	0.68	98
10/17	15	23.00	100
10/21	20	52.00	50
10/24	20	92.00	150
10/28	20	38.00	93

TABLE 32. Effect of cadmium, zinc, and copper ions and NTA Chelates of the same on Metabolism of ^{14}C glucose by NTA degrading isolates. Metal ions present as 1 mg/l; NTA at 4 mg/l. All assays at 18C.

Isolate	Inoculum (10^4 cells/ml)	^{14}C Incorporation as Percent: Metal/chelate	Metal/control	Metal/NTA	Mean	Percent Inhibition	^{14}C Incorporation as Percent: Chelate control	NTA control
Cadmium								
NTA-T1	0.69	57	49	49	52	48	86	100
216	548.00	177	59	98	59*	41	34	61
216	91.60	21	15	28	15*	85	70	51
Zinc								
NTA-T1	0.69	62	49	49	53	47	79	100
216	548.00	81	83	137	82+	18	103	61
216	91.60	118	92	179	92*	8	78	51
Copper								
NTA-T1	0.69	14	8	8	10	90	58	100
216	548.00	11	6	10	9	91	54	61
216	91.60	17	10	19	10*	90	58	51

* Percent control only

+ NTA omitted from calculation

TABLE 33. Effect of copper, cadmium, zinc, and mercury ions and NTA-metal chelates on Microbial Metabolism. (Three Replicates per Treatment, all data as $\bar{X}$, s.d.).

Exposure	Cell number at 22 hr (x 10^6)	Respiration (% O_2 depletion/min.) at indicated time: 0 (Before exposure)	0 (After exposure)	2.5 hr.	22 hr.	22 hr.*	Metals (u mole) Supernatant 0	Supernatant 22 hr.	Cells 22 hr.
100 mg/l Metal, 500 mg/l NTA									
Cu	26, 2	1.1, 0.2	0	0.8, 0.1	3.0, 1.2	----------	78.6	54.3, 6.2	1.7, 0.1
Cu/NTA	158, 41	1.0, 0	1.0, 0	2.4, 1.3	40.0, 3.8	----------	78.6	49.6, 2.8	1.1, 0.1
NTA	122, 23	1.1, 0.1	1.1, 0.1	2.7, 0.3	38.0, 4.0	----------			
Control	85,	1.4, 0.2	--------	2.8,	32.0,	----------			
Cd	483,138	1.9, 0.4	0,	0.5, 0.1	5.9, 1.8	6.2, 2.0	44.5	13.4, 2.0	0.25,0.03
Cd/NTA	486, 29	2.3, 0.3	2.1, 0.3	2.5, 0.5	8.2, 2.0	9.2, 2.5	44.5	15.9, 0.2	0.09,0
NTA	302,167	2.4, 0.1	2.4, 0.1	3.3, 0.2	2.8, 0.2	17.8, 0.8			
Control	280, 60	1.8, 0.2	--------	2.3, 0.4	4.8, 1.5	15.7, 3.4			
Zn	370, 89	0.9, 0.1	0	0.3, 0.1	3.8, 0.8^{a}	----------	76.4	28.0, 3.2^{a}	66.5, 3.3^{a}
Zn/NTA	153, 58	1.2, 0.2	1.2, 0.2	16.6, 0.5	24.2, 6.0^{a}	----------	76.4	66.6, 4.9^{a}	13.4, 1.1^{a}
NTA	402,207	1.2, 0.3	1.3, 0.3	9.4, 3.1	12.2, 7.8^{a}	----------			
Control	577,264	1.1, 0.1	--------	5.0, 0.5	20.0,10.8^{a}	----------			
Hg	20, 4	2.9, 0.2	0	0	0.1, 0.1	0.1, 0.1	24.9	16.2, 2.7	4.6, 0.6
Hg/NTA	11, 6	2.7, 0.1	0	0	0.2, 0.1	0.2,	24.9	20.2,	0.8,
NTA	580,183	2.8, 0.2	2.8, 0.2	2.8, 0.5	4.3, 0.7	7.4, 2.2			
Control	185, 21	3.3, 0.3	--------	3.4, 0.4	6.4, 0.9	9.0, 0.5			
1 mg/l Metal, 5 mg/l NTA									
Cu	32, 7	0.8, 0.1	0	0.5, 0.2	0.4, 0.1	1.7, 0.2	78.0	44.7,11.0	7.5, 0.9^{b}
Cu/NTA	26, 3	1.2, 0.4	1.3, 0.4	2.2, 0.2	3.3, 0.8	3.8, 0.7	78.0	74.9, 4.7	2.6, 2.3^{c}
NTA	60, 19	2.1, 0.2	1.4, 1.1	3.7, 0.5	4.2, 0.5	6.8, 1.5			
Control	44, 6	0.8, 0.2	--------	2.3, 0.1	2.7, 0.8	4.7, 3.5			

[a] 65 hr.

[b] mg dry wgt cells = 1.4, 0.2; u moles metal/mg dry weight = 5.40, 0.77

[c] mg dry wgt cells = 3.6, 0.2; u moles metal/mg dry weight = 1.09, 0.19

*following nutrient addition

TABLE 34. Concentration of Selected Chemical Species in Ecosystem Stream Water; July 13-October 6, 1976. Data in mg/l but for Mn, Fe, Zn which are ug/l. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA.

Stream	CONCENTRATION													
	NO_3-N	NO_2-N	NH_3-N	PO_4-P	SiO_2	pH	$SO_4^=$	Cl-	Total Alkalinity	Ca	Mg	Mn	Fe	Zn
I														
max	3.14	.012	.078	.07	18.7	7.8	17.9	12.0	71	27.2	19.5	28.3	591	9.3
min	1.79	.002	.002	.02	15.2	7.5	14.1	8.0	56	11.2	5.4	0	87	0
$\bar{x}$	2.74	.006	.014	.04	17.1	7.6	15.4	9.4	60	16.9	10.4	16.6	221	1.4
n	78	77	61	75	18	28	18	19	19	21	21	21	21	21
II														
max	3.19	.014	.073	.07	19.1	8.1	18.0	11.0	64	28.1	15.2	29.2	590	9.3
min	1.79	.002	.002	.02	15.4	7.5	14.6	8.0	54	11.1	5.5	0	91	0
$\bar{x}$	2.78	.006	.010	.04	17.2	7.7	15.8	9.8	60	17.3	10.3	16.9	215	2.2
n	78	77	61	75	18	27	18	18	19	21	21	21	21	21
III														
max	3.19	.011	.076	.07	19.0	7.8	17.8	11.0	66	26.9	19.6	29.2	642	10.0
min	1.79	.002	.002	.02	14.8	7.6	14.2	8.0	57	11.1	5.5	0	81	0
$\bar{x}$	2.78	.006	.009	.04	17.2	7.8	15.5	9.8	61	17.2	10.6	18.0	228	4.0
n	78	77	61	75	18	28	18	18	19	21	21	21	21	21
IV														
max	3.19	.012	.076	.07	19.0	7.8	17.8	12.0	65	25.5	19.6	26.8	607	11.6
min	1.79	.002	.002	.02	15.2	7.0	13.2	9.0	56	11.3	5.5	0	84	0
$\bar{x}$	2.80	.006	.011	.04	17.0	7.7	15.4	10.2	61	16.7	10.4	15.8	221	2.4
n	78	77	61	75	18	28	18	19	19	21	21	21	21	21

TABLE 35. Concentrations of NTA and copper in ecosystem stream water; July 13-October 6, 1976. NTA reported in mg/l; cooper in ug/l. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA.

Compound	Concentration in Stream			
	I	II	III	IV
NTA (GC analysis)				
n	8	60	9	60
$\bar{x}$	0.06	1.99	0.06	2.07
s.d.	0.07	0.75	0.05	1.00
c.v.	116	38	93	49
$S\bar{x}$	0.02	0.10	0.02	0.13
$S\bar{x}$(% mean)	41	5	31	6
max.	0.19	4.10	0.15	4.25
min.	0.01	0.22	0.01	0.02
NTA (Zinc-Zincon)				
n		81		77
$\bar{x}$		1.88		1.86
s.d.		0.44		0.63
c.v.		23		34
$S\bar{x}$		0.05		0.07
$S\bar{x}$(% mean)		3		4
max.		2.65		3.30
min.		0		0
Copper				
n	21	20	20	20
$\bar{x}$	3.6	31.1	29.2	3.9
s.d.	3.8	10.7	13.3	3.8
c.v.	105	35	46	99
$S\bar{x}$	0.8	2.4	3.0	0.9
$S\bar{x}$(% mean)	21.8	7.7	10.2	21.8
max.	15.0	49.0	55.4	9.3
min.	0	0	0	0

TABLE 36. Comparison of Concentration of Cu, Fe, Zn, Mn, and Mg in Algal Biomass from Ecosystem Streams at a given Sampling Time. Results of Scheffe Test (S) or Kurskal-Wallis Test (K), both at P = 0.05, are indicated by grouping streams between which no significant differences existed in parenthesis. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA.

Collection & Date		(Stream between which no significant difference exists):				
		Cu	Fe	Zn	Mn	Mg
1	7/13	(I,II,III,IV) S	(I,II,III,IV) S	(I,II,III)(II,III,IV) S	(I,II)(II,III,IV) K	(I,II,III,IV) K
2	7/20	(I,II,IV) S	(I,II,IV) S	I(II,IV) S	(I,II,IV) S	(I,II,IV) S
3	7/28	(I,II,IV) S	(I,II,IV) S	(I,II,IV) S	(I,II,IV) S	(I,II,IV) S
4	8/11	(I,II,III), (III,IV) K	(I,II,III,IV) S	(II,III)(III,IV) S	(I,II,III)(I,II, IV) K	(I,II)(I,III, IV) S
5	8/24	---	(I,II,IV)III S	---	(I,II)(I,III), (II,IV) K	(I,II,III,IV) S
6	9/7	(I,II,IV)(II, III) K	(I,II,IV)(I, III,IV) K	(I,II,III)(I,II,IV) K	(I,II,III,IV) S	(I,II,IV)(III,IV) S
7	9/21	(I,II,IV)III S	(I,IV)(II,III) S	(I,II,IV),III K	(I,II,III,IV) K	(I,IV)(II,III) (II,IV) K
8	10/6	(I,II,IV) S	(I,II,IV) K	(I,IV)(II,IV) K	I,II,IV S	(I,II)(I,IV) K

TABLE 37. Comparison of Concentration of Cu, Fe, Zn, Mn and Mg om *Anacharis* from Ecosystem Streams at a given Sampling Time. Results of Scheffe Test (S) or Kruskal-Wallis Test (K), both at P = 0.05, are indicated by grouping streams between which no significant differences existed in parenthesis. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA.

Collection & Date		(Streams between which no significant difference exists):									
		Cu		Fe		Zn		Mn		Mg	
1	7/13	(I,II)(I, III, IV)	K	(I,II,III)(II,III, IV)	S	(I,II,III)(I,III, IV)	K	(I,II,III,IV)	K	(I,II,III,IV)	S
2	7/20	(I,II,IV)(I,III)	K	(I,II,IV)III	S	(I,II,III)IV	K	(I,II,IV)(I,III, IV)	S	(I,II,III,IV)	K
3	7/28	(I,III)(II,III, IV)	K	(I,II,IV)III	S	(I,II,III,IV)	K	(I,II,IV)(I,III)	S	(I,II,IV)III	S
4	8/11	(I,IV)(II,IV)	K	(I,II)(II,IV)	S	I(II,IV)	K	I(II,IV)	S	(I,II,IV)	S
5	8/24	(I,II)	S	(I,II)	S	I,II	S	(I,II)	K	(I,II)	S
6	9/7	(I,IV)II	S	(I,II,IV)	S	(I,II IV)	K	(I,II,IV)	S	(I,II,IV)	K
7	9/21	(I,IV)II	S	(I,IV)(II,IV)	S	I(II,IV)	S	(I,II,IV)	S	(I,II.IV)	S
8	10/6	(I,II)(I,IV)	K	(I,II,IV)	K	(I,IV)(II,IV)	K	(I,II,IV)	K	(I,IV)(II,IV)	S

TABLE 38. Comparison of Concentrations of Cu, Fe, Zn, Mn, and Mg in *Lemna* from Ecosystem Streams at a given Sampling Time. Results of Scheffe Test (S) or Kruskal-Wallis Test (K), both at P = 0.05, are indicated by grouping streams between which no significant differences existed in parenthesis. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA.

Collection & Date		(Streams between which no significant difference exists): Cu		Fe		Zn		Mn		Mg	
1	7/13	(I,II,III)(II,III,IV)	K	(I,II,III,IV)	S	(I,II,III,IV)	K	(I,II)(I,IV)(III,IV)	S	(I,II,III)IV	S
2	7/20	(I,II)(II,III)(I,IV)	S	(I,II,IV)(III,IV)	K	(I,II)(II,III,IV)	S	(I,II,IV)(III,IV)	S	(I,II,III,IV)	S
3	7/28	(I,II,III,IV)	S	(I,II,IV)III	K	---		(I,II,IV)(II,III)	K	(I,II,III)(I,II,IV)	S
4	8/11	(I,II,IV)(II,III,IV)	K	(I,II,III)(II,III,IV)	K	(I,III)(II,III,IV)	S	(I,II,III)(II,III,IV)	K	(I,II,III)(I,II,IV)	K
5	8/24	(I,III,IV)(II,III)	S	(I,II,III,IV)	K	(I,III,IV)(II,III)	K	(I,II)(III,IV)	S	(I,II,III,IV)	S
6	9/7	(I,II,III,IV)	S	(I,III)(II,III,IV)	S	(I,II,III)(II,III,IV)	S	I,III(II,IV)	S	(I,II,IV)(I,III)	S
7	9/21	(I,II,III,IV)	K	(I,II,III,IV)	S	(I,III)(II,III,IV)	K	(I,IV)II,III	S	(I,II,III)(II,III,IV)	K
8	10/6	(I,II)(I,III)	K	(I,III)(II,III)	K	I(II,III)	S	(I,III)(II,III)	S	(I,II)III	S

TABLE 39. Comparison of Concentrations of Cu, Fe, Zn, Mn, and Mg in *Planaria* from Ecosystem Streams at a given Sampling Time. Results of Scheffe Test (S) or Kruskal-Wallis Test (K), both at P=0.05, are indicated by grouping streams between which no significant differences existed in parenthesis. Stream I-Control, II-NTA/Cu, III-Cu, IV-NTA.

Collection & Date		(Streams between which no significant difference exists):									
		Cu		Fe		Zn		Mn		Mg	
1	7/13	(I,II,III)(III,IV)	K	(I,II,III,IV)	S	(I,II,III,IV)	S	(I,II,III,IV)	S	(I,II,III,IV)	S
2	7/20	(I,II,IV)(I,II)(II,IV)	S	---		---		---		---	
3	7/28	(I,II,IV)	S	(I,II,IV)	K	(I,II,IV)	S	(I,II,IV)	S	(I,II,IV)	S
4	8/11	(I,II,IV)	K	(I,II)IV	S	(II,IV)	S	(I,II,IV)	S	(I,IV) II	K
5	8/24	(I,II,IV)	S	---		---		(I,II,IV)	S	(I,II,IV)	S
6	9/7	(I,II,IV)	K	(I,II,IV)	K	(I,II,IV)	S	(I,II,IV)	S	(I,II)(II,IV)	S
7	9/21	(I,III)	S	(I,III)	S	(I,III)	S	(I,III)	S	(I,III)	K
8	10/6	(I,II,IV)	S	(I,II)(II,IV)	K	(I,II,IV)	S	(I,II,IV)	S	(I,II,IV)	S

TABLE 40. Grand Mean of Metal Concentrations in Tubificid Worms from Collections 3-8.

Metal	Concentrations (μg/g; n, $\bar{x} \pm$ s.d.) for Material from Stream:				Statistical test results (Streams between which no significant difference exists)	
	I	II	III	IV		
	Control	Cu/NTA	Cu	NTA		
Cu	10, 44± 18	11, 96± 49	10, 142± 111	4, 56± 40	(I,IV)(II,III,IV)	K*
Fe	9,5704±2816	10,5052±3043	9,5676±5167	5,15266±17723	(I,II,III,IV)	K
Zn	8, 219± 247	8, 358± 277	7, 335± 285	5, 186± 108	(I,II,III,IV)	S**
Mn	8, 227± 132	10, 236± 144	10, 199± 174	4, 238± 133	(I,II,III,IV)	S
Mg	8,1377± 563	12,1624± 420	9,1562± 644	4, 1050± 231	(I,II,III,IV)	S

*K = Kruskal-Wallis test

**S = Scheffe test

TABLE 41. Cumulative Mean Concentration of Metal Ions in the Water in Ecosystem Streams; August 11 - October 6, 1976. Data in μg/l for all elements but Mg which is in mg/l.

Metal	Col-lec-tion	n	Cumulative Concentration in water ($\bar{x}$ ± s.d.) in Stream:			
			I	II	III	IV
			Control	Cu/NTA	Cu	NTA
Cu*	4	4	8.25 ± 6.50	28.51 ± 8.76	21.09 ± 6.28	8.14 ± 6.29
	5	8	6.63 ± 4.60	29.30 ± 6.38	19.22 ± 10.86	6.07 ± 4.85
	6	14	5.93 ± 3.47	26.90 ± 9.36	21.15 ± 10.41	5.61 ± 3.60
	7	16	6.44 ± 3.97	28.80 ± 10.46	23.32 ± 11.37	6.18 ± 4.15
	8	19	6.45 ± 3.72	30.14 ± 10.59	26.42 ± 13.40	6.22 ± 3.88
Fe	4	4	247.00 ± 57.46	236.75 ± 29.34	248.90 ± 27.18	276.06 ± 85.29
	5	8	255.93 ± 44.90	244.60 ± 33.35	246.58 ± 41.03	254.52 ± 63.90
	6	14	225.53 ± 55.99	218.39 ± 45.46	233.30 ± 65.11	226.27 ± 62.49
	7	15	229.01 ± 55.61	223.43 ± 47.96	238.01 ± 65.34	230.71 ± 62.63
	8	18	211.54 ± 65.53	204.14 ± 62.67	218.40 ± 75.52	211.93 ± 72.37
Zn*	4	4	3.00 ± 0.00	4.71 ± 3.07	4.01 ± 1.98	5.52 ± 4.13
	5	8	4.73 ± 3.21	5.83 ± 4.04	5.16 ± 2.69	5.95 ± 3.45
	6	14	4.19 ± 2.54	4.71 ± 3.27	4.23 ± 2.26	4.84 ± 2.91
	7	16	4.13 ± 2.39	4.56 ± 3.03	4.17 ± 2.12	4.72 ± 2.75
	8	19	3.95 ± 2.22	4.31 ± 2.87	3.98 ± 1.99	4.34 ± 2.69
Mn*	4	4	23.23 ± 4.87	22.51 ± 4.06	23.36 ± 5.58	21.37 ± 7.65
	5	8	18.73 ± 7.35	18.33 ± 7.29	19.34 ± 8.09	17.18 ± 7.81
	6	14	18.95 ± 7.21	18.59 ± 6.95	19.15 ± 7.51	17.79 ± 7.29
	7	16	17.79 ± 7.62	17.33 ± 7.44	18.22 ± 7.83	16.68 ± 7.57
	8	19	16.66 ± 7.63	16.70 ± 7.00	17.90 ± 7.23	16.26 ± 7.13
Mg	4	4	10.51 ± 2.56	10.41 ± 2.37	10.14 ± 2.10	9.46 ± 2.23
	5	8	12.42 ± 4.35	12.23 ± 4.15	12.81 ± 4.99	11.78 ± 4.63
	6	14	10.96 ± 4.04	11.05 ± 3.80	11.19 ± 4.58	10.91 ± 4.00
	7	16	10.81 ± 3.79	10.92 ± 3.57	11.07 ± 4.29	10.74 ± 3.75
	8	19	10.48 ± 3.58	10.50 ± 3.42	10.62 ± 4.06	10.39 ± 3.57

*Lower limit of detection: Cu - 5 ug/l, Zn-3 ug/l, Mn-5 ug/l

TABLE 42. Concentration Factors [Metals in Biomass of indicated Sample/Metal in Water]. Data shown are averages of 3-5 collections for which factors were calculated.

Sample and Metal	Sample PPB/water PPB (n, $\bar{x} \pm$ S.D.) for stream:				Statistical test results:* (streams between which no significant difference exists)	
	I	II	III	IV		
	Control	Cu/NTA	Cu	NTA		
ALGAE						
Cu	4, 9449± 5908	4, 2903± 1945	4, 2293± 8110	4, 31719±46946	(I,II,IV)(I,III,IV)	K
Fe	5, 44494± 23024	5, 41568± 21720	5, 52270± 17335	5, 34636±10057	(I,II,III,IV)	S
Zn	3, 21430± 5693	4, 12794± 5530	3, 24179± 5246	4, 14010± 3000	(I,II,III,IV)	S
Mn	5, 59052± 32061	5, 66238± 26671	5, 44501± 20609	5, 96982±49452	(I,II,III,IV)	S
Mg	5, 208002± 65566	5, 231367±109119	5, 268009± 70456	5, 270312±77072	(I,II,III,IV)	S
ANACHARIS						
Cu	5, 75384± 58548	5, 16783 ± 9699	106686	4, 50464±41766	(I,II,IV)	S
Fe	5, 10808± 1684	5, 9050 ± 2133	19719	4, 8820± 1434	(I,II,IV)	S
Zn	5, 71193± 47424	5, 32871 ± 20114	81197	4, 40801±33413	(I,II,IV)	S
Mn	5, 12465± 1365	5, 10722 ± 2299	21706	4, 12416± 2350	(I,II,IV)	S
Mg	5, 295908± 24486	5,283717 ± 30258	--	4, 332534±66311	(I,II,IV)	S
LEMNA						
Cu	5, 91597± 75213	5, 16992 ± 11750	5, 31507± 20509	4, 108210±71787	(I,II,III,IV)	S
Fe	5, 21072± 16645	5, 19839 ± 9433	5, 17538± 10911	4, 40730±33239	(I,II,III,IV)	S
Zn	5, 66522± 28899	5, 25884 ± 17468	5, 36586± 20761	4, 31811±12179	(I,II,III,IV)	S
Mn	5, 46647± 6547	5, 58173 ± 10230	5, 37644± 16502	4, 53554±11115	(I,II,III,IV)	S
Mg	5, 278492±122209	5,311459 ± 33592	5, 338253±112813	4, 374494±97617	(I,II,III,IV)	S
PLANARIA						
Cu	5, 7596± 3660	4, 3995 ± 1791	3428	4, 13495± 7198	(I,II)(I,IV)	S
Fe	4, 2627± 1158	3, 9666 ± 12837	2096	3, 1973± 1486	(I,II,IV)	S
Zn	3, 98935± 40564	3, 70527 ± 10507	283211	3, 77193±15343	(I,II,IV)	S
Mn	5, 2255± 1104	4, 3057 ± 1579	1754	4, 2313± 697	(I,II,IV)	S
Mg	5, 85215± 44395	4,128992 ± 24849	98284	4, 117597± 2918	(I,II,IV)	S
TUBIFEX						
Cu	5, 6167± 2966	5, 3400 ± 1517	5, 6878± 2884	3, 8710± 4782	(I,II,III,IV)	S
Fe	5, 25181± 9773	5, 22130 ± 10646	5, 21258± 16864	4, 42070±49169	(I,II,III,IV)	S
Zn	4, 56509± 62589	4, 79702 ± 57900	3, 88180± 54438	3, 29407±23588	(I,II,III,IV)	S
Mn	4, 14583± 5198	4, 14563 ± 7096	4, 12635± 9150	2, 18472± 9484	(I,II,III,IV)	S
Mg	4, 14690± 36473	5,157204 ± 20156	5, 135420± 42813	3, 109952±14832	(I,II,III,IV)	S

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*Scheffe multiple range test used except for Algae-Copper, where the Kruskal-Wallis test was used.

TABLE 43. Concentration Factors [Metal in Planaria/Metal in Algae]. Data shown are averages of 4-7 collections for which factors were calculated.

Metal	Planaria PPB/Algae PPB (n, $\bar{x} \pm$ s.d.) for Stream:				Scheffe Test Results: (Streams between which no significant differences exists)
	I	II	III	IV	
	Control	Cu/NTA	Cu	NTA	
Cu	7, 0.93±0.97	5, 0.93±0.41	0.16	5, 0.69±0.33	(I,II,IV)
Fe	5, 0.07±0.03	4, 0.20±0.24	0.02	4, 0.13±0.17	(I,II,IV)
Zn	4, 4.44±3.15	4, 6.05±2.80	9.49	4, 5.30±1.27	I, (II,IV)
Mn	6, .05± .03	5, .05± .02	.03	5, .03± .02	(I,II, IV)
Mg	6, 0.62±0.43	5, 0.74±0.37	0.25	5, 0.46±0.16	(I,II,IV)

TABLE 44. Concentrations of Copper and NTA in Microcosms for Sediment Studies (10/13-12/17/76)

Microcosm	Concentrations: $\bar{x}$ ±s.d. (n)	
	Cu (ug/l)	NTA (mg/l)
Control	4.16 ± 3.56 (38)	
Copper	23.88 ± 10.92 (41)	
Copper/NTA	30.20 ± 7.60 (41)	1.77 ± 0.33 (37)

TABLE 45. Concentration of Copper (ng/g) in Sediments Exposed to Copper or Copper/NTA.

Depth	Date	Control			Copper			Copper/NTA			Results of Statistical Tests: (Treatments between which no significant difference exists)
		n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	
Surface	10/29	2	356.5	42.5	2	539.5	238.3	2	371.5	70.0	
	11/8	2	401.0	7.0	2	450.5	62.9	2	819.5	731.2	
	11/24	2	376.5	195.9	2	453.0	59.4	2	636.0	2.8	
	12/17	4	329.2	308.0	4	590.2	280.0	3	661.0	255.1	
2 cm	10/29	2	237.5	84.1	2	448.0	87.7	2	393.5	143.5	
	11/8	2	413.5	178.9	2	564.5	307.6	2	440.5	146.4	
	11/24	2	208.5	28.3	2	267.5	27.6	2	309.5	38.9	
	12/17	4	142.4	39.7	4	324.8	112.0	4	406.0	60.0	
5 cm	10/29	2	205.5	115.3	2	344.0	80.6	2	354.5	129.4	
	11/8	2	339.5	3.5	2	314.5	31.8	2	346.5	62.9	
	11/24	2	282.5	48.8	2	250.5	3.5	2	299.5	3.5	
	12/17	4	214.0	93.7	4	254.0	131.0	4	343.0	126.0	
	For the Experiment:										
Surface		4	365.8	30.4	4	508.5	68.4	4	622.0	185.3	(C,Cu) (Cu, Cu/NTA)*
2 cm		4	250.4	115.8	4	401.2	132.4	4	387.4	55.6	(C, Cu, Cu/NTA)*
5 cm		4	260.4	63.0	4	290.8	46.1	4	335.9	24.7	(C, Cu, Cu/NTA)*
	Statistical Test Results:										
(Depths between which no significant differences exists)			(S, 2,5)*			(S,2)(2,5)*			(S,2)(2,5)**		

* Scheffe Test, P = 0.05
** Kruskal-Wallis Test, P = 0.05

TABLE 46. Concentration of Iron, Manganese, and Zinc (all in µg/g) in Sediments on 12/17 after 65 days Exposure to Copper or Copper/NTA.

Depth	Control			Copper			Copper/NTA			Results of Statistical Tests: (Treatments between which no significant differences existed)
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	
Iron										
Surface	4	27.6	16.6	4	43.5	14.8	3	46.6	23.5	(C,Cu,Cu/NTA)*
2 cm	4	26.8	11.4	4	60.0	21.2	4	63.8	13.8	C (Cu,Cu/NTA)*
5 cm	4	43.3	21.1	4	40.8	27.6	4	52.3	22.3	(C,Cu,Cu/NTA)*
Manganese										
Surface	4	45.9	21.8	4	34.5	11.9	3	26.5	14.2	(C,Cu,Cu/NTA)*
2 cm	4	28.0	4.4	4	38.8	8.4	4	33.2	11.3	(C,Cu,Cu/NTA)*
5 cm	4	42.6	14.8	4	38.0	6.9	4	32.5	12.0	(C,Cu,Cu/NTA)*
Zinc										
Surface	4	5.56	2.83	4	5.20	0.84	3	4.92	1.70	(C,Cu,Cu/NTA)*
2 cm	4	2.84	0.91	4	4.47	1.16	4	4.07	0.87	(C,Cu,Cu/NTA)*
5 cm	4	3.98	0.86	4	4.59	4.50	4	2.91	0.75	(C,Cu,Cu/NTA)**

* Scheffe Test, P = 0.05
** Kruskal-Wallis Test, P = 0.05